REVIEW

Ovarian clear cell adenocarcinoma: a continuing enigma

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Ovarian clear cell adenocarcinomas (OCCAs) account for <5% of all ovarian malignancies. Compared to other epithelial ovarian cancer (EOC) subtypes, when at an advanced stage, they are associated with a poorer prognosis and are relatively resistant to conventional platinum-based chemotherapy. By contrast, early-stage clear cell ovarian cancer carries a relatively good prognosis. Hence, there is a need to improve our understanding of its pathobiology in order to optimise currently available treatments and develop new therapeutic strategies. This review summarises the currently available literature regarding the pathogenesis of OCCA, its molecular genetic features and postulated molecular mechanisms that underlie its chemoresistant phenotype. Marked similarities with clear cell carcinomas of the kidney and endometrium have been noted by some investigators, raising interesting possibilities regarding novel therapeutic approaches. Unfortunately, most studies on OCCA have hitherto been hampered by insufficient sample sizes, leaving many key issues unresolved. It is envisaged that in the future, high-resolution genomic and gene-expression microarray studies incorporating larger sample sizes will lead to the characterisation of the key molecular players in OCCA biology, which may potentially lead to the identification of novel targets for therapeutic development.

Ovarian clear cell adenocarcinomas (OCCAs) account for <5% of all ovarian malignancies, and 3.7–12.1% of all epithelial ovarian carcinomas (EOCs).[1–5] They were originally described by Schiller[6] in 1939, who coined the term ''mesonephroma'' to describe an ovarian neoplasm comprised of clear and hobnailed cells with an immature glomerular pattern. Subsequently, it was observed that Schiller's original descriptions actually included two distinct populations: a highly malignant germ cell tumour occurring in younger women, and another tumour of epithelial origin with a less aggressive phenotype, which was formally designated as OCCA in 1973 by the World Health Organization.[7–9]

CLINICOPATHOLOGICAL CHARACTERISTICS

When compared with their serous counterparts, a greater proportion of OCCA tumours present as early-stage (I–II) tumours, are often associated with a large pelvic mass, which may account for their earlier diagnosis, and rarely occur bilaterally.[10] An increased incidence of vascular thrombotic events and hypercalcaemia is seen in patients with OCCA,[3 11] with a higher frequency of lymph node metastases also being noted for these patients among the non-serous types of EOCs.[12]

Like most other EOCs, nulliparity is a risk factor for OCCA, whereas tubal ligation and, more tenuously, smoking may be protective.[13] A family history of breast or ovarian cancer does not seem to confer predisposition to OCCA.[13] An association between OCCA and endometriosis has been reported in 25–58% of cases in various studies,[2 3 11] but these figures might actually be gross underestimations, as pre-existing endometriosis may be obliterated by the tumour or not sampled for histological evaluation during surgery. Indeed, it may be postulated that the protective effect of tubal ligation in the development of OCCA may stem from the resulting prevention of retrograde menstruation, and hence the subsequent development of endometriosis.

Histopathologically, the tumour is comprised of large cuboidal, hobnailed or flattened epithelial cells containing abundant clear cytoplasm lining tubules and cysts, and growing in solid/tubular or glandular patterns.[9] In addition, an oncocytic (oxyphilic) variant of OCCA also exists, where the tumour cells have eosinophilic rather than clear cytoplasm.[14] Immunohistochemical studies on OCCA have usually shown positive staining for the proapoptotic protein Bcl-2-associated death protein (BAX), p21 and cyclin E, and weak staining for p53, cyclin A and HER-2 compared with other EOC subtypes.[15–17] Poor prognostic factors in OCCA include young age (<60 years), advanced stage and the presence of vascular invasion, whereas the presence of a predominantly (>75%) papillary or tubulocystic morphological pattern independently predicts a better prognosis.[4 11] With regard to the histological grading of OCCA tumours, no formal consensus exists.[18 19] Although the vast majority of OCCA tumours are considered to be of high grade, histological grading of these tumours either by conventional architectural and cytological criteria or by the grading criteria devised by Shimizu and colleagues (a grading system for invasive ovarian cancer based on the Nottingham grading criteria for breast cancer, which incorporates a quantification of the predominant architectural pattern, degree of

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Abbreviations: ABCF2, ATP-binding cassette transporter F2; BAX, Bcl-2-associated X protein; Bcl-2, B cell lymphoma-2; EOC, epithelial ovarian carcinoma; hMLH1 and hMSH2, mutL homolog 1 and mutS homolog 2; HNF-1, hepatocyte nuclear factor-1; MRP, multidrug resistance associated protein; OCCA, ovarian clear cell adenocarcinoma; P-gp, P-glycoprotein; PTEN, phosphatase and tensin homologue; TMS-1/ASC, target of methylation-induced silencing-1/apoptosis-associated speck-like protein; WT1, Wilms' tumour suppressor gene

nuclear pleomorphism and mitotic activity in each tumour) has shown no prognostic value.[20 21]

The general consensus from numerous retrospective studies is that, at advanced stages (stage III/IV), OCCA carries a poor prognosis with insensitivity to platinum-based chemotherapy relative to serous and other non-clear cell EOCs.[2 5 22–27] Intriguingly, in the largest retrospective study of 254 patients with OCCA to date by Takano *et al*, stage IA tumours were found to have a good prognosis equivalent to their serous counterparts, whereas stage III and IV tumours had a poorer prognosis.[27] A significantly worse prognosis in stage III OCCA compared with equivalent stage serous ovarian cancer has also recently been demonstrated by Mizuno *et al*.[28] In addition, Takano's study observed a treatment response in only up to 32% of patients with OCCA, with no difference in outcome when different platinum-containing chemotherapeutic regimens were compared.[27] Hence, there is a need to improve our understanding of its pathobiology in order to optimise currently available treatments and develop new therapeutic strategies.

DEVELOPMENT AND PROGRESSION OF OCCA

Very little is known about the pathobiology of OCCA. Between 5% and 10% of ovarian cancers are associated with endometriotic lesions in which there is a predominance of clear and endometrioid cell subtypes,[29–31] suggesting that both tumour types may arise in endometriosis. There has been little molecular evidence to support a developmental origin for OCCA from endometriotic lesions until recently, when Sato *et al*[32] demonstrated that loss of heterozygosity events were common in OCCA and synchronous endometriotic lesions. Kato *et al*[33] examined 30 clear cell tumours (26 malignant, 3 borderline and 1 benign) and 40 endometriotic cysts for immunohistochemical expression of hepatocyte nuclear factor-1 beta (HNF-1β) in the nucleus. HNF-1β had previously been shown to be upregulated and overexpressed in OCCA cell lines[34] and has been implicated in mediating apoptotic escape in tumour cells.[34] All of the 30 clear cell tumours showed nuclear expression of HNF-1β, whereas other EOC subtypes rarely expressed it. In addition, distinct nuclear immunostaining for HNF-1β was detected in endometriotic epithelia associated with clear cell tumours. Furthermore, 40% of endometriotic cysts without neoplastic changes also expressed HNF-1β, mainly in areas exhibiting inflammatory atypia. Hence, early differentiation into the clear cell lineage may take place in ovarian endometriosis, leading to its frequent association with OCCA.

Mutations in p53 are a common event in tumorigenesis, and have been noted in various EOC subtypes, being particularly common in serous ovarian carcinomas but conspicuously absent in OCCA.[15 35] This implies that other antiapoptotic mechanisms are likely to be involved in the development of OCCA. Aberrant methylation resulting in transcriptional silencing of the target of methylation-induced silencing (TMS)-1/apoptosis-associated speck-like protein (TMS-1/ASC), a member of the caspase recruitment domain family of proapoptotic mediators, has been observed at a significantly high frequency in OCCA tumours. This indicates a mechanism for apoptotic escape in tumour development, which may have implications for drug resistance in OCCA as well.[36] Another prominent candidate thought to play an important role in the pathogenesis of OCCA is the PTEN tumour suppressor gene. Activation of PTEN, a lipid phosphatase in the Akt(phosphoinositide 3 kinase) pathway, results in the inhibition of cyclin D1 (CCND1) and p27, thus blocking cell cycle progression.[37 38] In addition, PTEN may also be involved in the regulation of cell migration, spreading and focal adhesion.[39 40] Loss of PTEN expression has been noted in 40% of early-stage OCCA, suggesting that PTEN inactivation may be an early event in OCCA development.[41]

The Wilms' tumour suppressor gene (WT1) encodes a zinc finger transcription factor, which binds to GC-rich sequences and functions as a transcriptional activator or repressor for many growth factor genes.[42] Although WT1 is expressed in serous adenocarcinomas of the ovary, its expression is lacking in OCCA.[43] The WT1 promoter is significantly methylated in OCCA compared with serous adenocarcinoma, and a significant correlation between methylation and mRNA expression status has been observed.[43] WT1 has been shown to suppress insulin-derived growth factor-I receptor gene transcription in breast cancer cells, and its inactivation may result in deregulated insulin-derived growth factor-I receptor gene expression and enhanced mitogenic activation.[44] In addition, the cell-adhesion molecule E-cadherin, which is down regulated in most epithelial malignancies, has also been identified as a transcriptional target of WT1.[45] Reduced expression of E-cadherin has been observed in OCCA.[46] Hence, WT1 inactivation may play an important role in the development of OCCA.

Loss of CD44 splice control has also been observed in OCCA.[39 47] CD44 is a membrane glycoprotein and is the major cell-surface receptor of hyaluronate, a glycosaminoglycan which is present on the surface of human peritoneal cells. The presence of the CD44 isoform, CD44-10v, was associated with recurrence or death in 71% of the women with OCCA, whereas only 18% of the women experienced recurrence or died of disease when the isoform was not expressed in the primary tumour.[48] The CD44-10v isoform was also found to be absent in the contralateral non-affected ovaries, suggesting aberrant alternative mRNA splicing of CD44 in the development and progression of OCCA.

MOLECULAR GENETIC FEATURES

Suehiro *et al*[49] performed conventional comparative genomic hybridisation (cCGH) in 12 OCCAs, observing DNA copy number abnormalities in >20% of cases. These included increased copy numbers of 8q11–q13, 8q21–q22, 8q23, 8q24–qter, 17q25–qter, 20q13–qter and 21q22–qter, with reduced copy numbers observed on 19p. The increased copy numbers on 8q occurred more often in disease-free patients than in recurrent/non-surviving patients ($p<0.05$). However, increases in copy numbers of 17q25–qter and 20q13–qter occurred more frequently in recurrent/non-surviving patients than in disease-free patients ($p<0.05$). Furthermore, increases in copy numbers of 17q25–qter and 20q13–qter occurred together ($p<0.05$). Based on the results, Suehiro *et al* classified OCCA into two subtypes, one being cancer with an increase in copy numbers of 8q and the other being cancer with increases in copy numbers of 17q25–qter and 20q13–qter. Frequent gains of 8q in OCCA have also been observed by Osterberg *et al*[36] in a study comparing carboplatin-resistant and sensitive early-stage EOC using CGH. Interestingly, none of the genetic changes that were associated with clinical carboplatin resistance in the study were observed in the OCCA subset. Gains of 8q are a commonly detected genetic aberration in ovarian tumours, with the candidate oncogene myelocytomatosis viral oncogene homologue localised to 8q24.1.[50] The overexpression of myelocytomatosis viral oncogene homologue has been noted in EOC.[51]

Using cCGH, Dent *et al*[52] analysed 18 OCCA tumours and found cytogenetic aberrations which were distinct from those of Suehiro *et al*. These included frequent amplifications of chromosomes 3 and 13q and deletions of 9p, 1p, 11q and 16p/q. Given the higher incidence of OCCA in Japanese women than in their Caucasian counterparts (15.3% vs 5%, respectively),[4 5] it is not clear whether these differences were a reflection of distinct molecular genetic pathways in the development of

OCCA, or indeed ovarian cancer as a whole, in Japanese women. Deletions of 9p have been noted in 41% of ovarian cancers across all histological grades,[53] and known tumour suppressor genes on 9p include the cyclin-dependent kinase inhibitor 2 genes known to encode the proteins p16 (INK4A), p15 (INK4B) and p14 (ARF).[54] These proteins act as tumour suppressor genes by regulating two growth control pathways that interact with Rb and p53.[55–57] Chromosome 9p deletions have also been noted in renal clear cell carcinomas.[58]

Gains of 17q21–q24 have shown significantly negative correlation with disease-free and overall survival in OCCA, including patients with stage I tumours.[59] Significantly, elevated expression of PPM1D and APPBP2 was observed among 15 candidate genes within the 17q21–q24 region, and correlated negatively with disease-free survival. The serine–threonine protein phosphatase Wip 1, which is encoded by the PPM1D gene, is thought to play an important role in tumorigenesis by acting as a negative feedback regulator of p53 and controlling the expression of other cell cycle regulatory proteins such as CCND1.[60] Hitherto, there is no functional link for APPB2, a microtubule-binding protein, to any cancer,[61] and its amplification may merely be secondary to its proximity to PPM1D, which is more likely to be the key amplicon driver.

A molecular signature which distinguishes OCCA from other histological types of ovarian cancer has been reported by Schwartz *et al*.[62] A total of 73 genes, expressed 2–29-fold higher in OCCA compared with each of the other ovarian carcinomas, were identified.[62] However, this study only included eight OCCA specimens and, unusually, observed a more than twofold increase in the expression of Her-2 compared with other EOC subtypes. More recently, a comparison of gene expression profiles of serous, endometrioid and clear cell subtypes of ovarian cancer with normal ovarian surface epithelium revealed a set of 43 common genes which appeared on the profile of each ovarian subtype.[47] This suggests that part of the process of malignant transformation in serous, endometrioid and clear cell subtypes of ovarian cancer may involve a common pathway. In addition, the profiles of OCCA were similar to those of renal and endometrial clear cell carcinomas, with the implication that certain molecular events may be common to clear cell tumours regardless of the organ of origin,[47] thus raising the possibility of crossover molecular targets in the treatment of these tumours.

MECHANISMS OF DRUG RESISTANCE IN OCCA

Cloven *et al*[63] examined in vitro drug response profiles for different histological subsets of EOC in over 5000 patients, including 102 OCCA tumours and 2660 serous tumours, and observed higher sensitivity to paclitaxel, cyclophosphamide and doxorubicin in OCCA cells when compared with serous ovarian carcinoma cells, with no significant differences in sensitivity observed for topotecan, carboplatin or cisplatin. In a separate study involving five OCCA cell lines, higher responses to SN-38, the active metabolite of the topoisomerase (topo) I inhibitor irinotecan, and paclitaxel were observed in 3 out of 5 cell lines.[64] All five cell lines were resistant to mitomycin-C and etoposide. Hence, the authors suggested that irinotecan and paclitaxel were more likely to be effective chemotherapeutic agents in OCCA. While there is no evidence for increased response to cyclophosphamide or doxorubicin in clinical studies, a prospective study by Ho *et al*[65] showed a significantly increased median survival in patients with OCCA treated with paclitaxel–platinum (paclitaxel with carboplatin or cisplatin) based chemotherapy (n = 16) as compared with patients treated with platinum-based chemotherapy (cisplatin/carboplatin and cyclophosphamide with or without doxorubicin) alone (n = 15). Combination chemotherapy using irinotecan with cisplatin or mitomycin C has also been reported to be effective in early trials and case reports of patients with OCCA.[32 66–68] However, given the small patient numbers in these studies, and the lack of prospective randomised controlled trials, these results should be interpreted with caution.

P-glycoprotein (P-gp) and multi-drug resistance associated protein (MRP), which actively transport substrates across membranes into vesicles and out of cells, are important multi-drug resistance factors.[69 70] The expression of MRP-1 in OCCA has been associated with cisplatin resistance in vitro.[64] However, in a subsequent comparative immunohistochemical study of P-gp and MRP expression in patients with stage III–IV OCCA and serous ovarian carcinomas, where platinum-based chemotherapy response rates were 14.6% and 72.2%, respectively, no difference was found in the expression levels of P-gp and MRP between responders and non-responders in both tumour types.[71] This suggests that the role of multidrug resistance genes in OCCA chemoresistance is less relevant in vivo. Interestingly, the study also revealed a significantly higher Ki-67 labelling index in responders than in non-responders in both tumour groups.[71] Furthermore, the labelling index in OCCA was significantly lower than in serous tumours. Higher rates of cell proliferative activity, of which the nuclear antigen Ki-67 is a reliable indicator, have been associated with improved response to chemotherapy.[72] Hence, the poor chemotherapy response rates in OCCA may ultimately be related to low proliferation activity in these tumours.

Microsatellite instability (MSI) is caused by defects in the DNA mismatch repair genes. In experimental systems, mismatch-repair-deficient cells are highly tolerant to the methylating chemotherapeutic drugs streptozocin and temozolomide, and, albeit to a lesser extent, to cisplatin and doxorubicin.[73] These drugs are therefore expected to be less effective on mismatch-repair-deficient tumours in humans. Cai *et al*[74] observed high-level MSI involvement in the development of a subset of OCCAs, and a strong correlation between alterations in the expression of hMLH1 and hMSH2 and the presence of MSI in these tumours. Significantly elevated mRNA levels of ERCC1 (excision-repair, complementing defective, in Chinese hamster-1) and XPB, two key genes involved in the nucleotide excision repair pathway and in vitro resistance of platinum-chemotherapy,[75] have also been observed in OCCA compared with other EOC subtypes.[76] Therefore, altering the expression of DNA repair genes may provide a possible mechanism of drug resistance against DNA damaging agents in OCCA.

In the gene expression study by Schwartz *et al*,[62] 3 of the 73 overexpressed genes in the eight OCCA samples analysed encoded the antioxidant proteins glutathione peroxidase 3, glutaredoxin and superoxide dismutase, prompting the suggestion that a high level of these and other antioxidant proteins may be responsible for chemoresistance in OCCA.[62] The expression of both annexin A4, which is associated with paclitaxel resistance,[77] and UDP glycotransferase 1 family, which is involved in SN-38 detoxification, has also been found to be increased in both ovarian and endometrial clear cell tumours in the study by Zorn *et al*.[47] Tsuda *et al*[78] subsequently performed genomic and gene expression array analyses on 30 OCCA and 19 serous EOC cases using a 10 816-element cDNA microarray platform. A significant increase in DNA and mRNA copy number of 12 genes, and a significant decrease in those of five genes were observed in OCCAs compared with serous tumours. Of the amplified genes, the ATP-binding cassette transporter F2 (ABCF2) gene on 7q35–36, which belongs to the ATP-binding cassette gene superfamily, was validated by real-time quantitative PCR and immunohistochemical analysis. Although both nuclear and cytoplasmic staining was seen, only high levels of cytoplasmic staining were shown to correlate significantly with non-response to

Table 1 Molecular characteristics of ovarian clear cell adenocarcinomas and their putative clinicopathological significance (see text for details)

Molecular characteristic	Molecular effect	Putative clinicopathological significance
HNF-1β upregulation	Apoptotic escape	Tumorigenesis
TMS-1/ASC methylation	Apoptotic escape	Tumorigenesis
PTEN loss	Activation of cell cycle progression	Tumorigenesis Metastasis
WT-1 reduced expression and promotor methylation	Deregulated IGF-IR gene expression and enhanced mitogenic activity	Tumorigenesis
	Reduced E-cadherin expression	Tumorigenesis/Metastasis
CD44 loss	Reduced cell adhesion	Metastasis
Myc amplification	Cell proliferation	Tumorigenesis
Chromosome 9p loss (CDKN2)	Loss of p53 and pRb activity	Tumorigenesis
Chromosome 17q21-q24 gain (PPM1D)	Inhibition of p53	Tumorigenesis
Loss of mismatch repair genes (hMLH1 and hMSH2)	Microsatellite instability	Drug resistance
ERCC1 and XPB upregulation	Impaired nucleotide excision repair	Platinum resistance
GPX3, GLRX and SOD2 (antioxidant proteins) upregulation	Resistance to oxidative damage	Drug resistance
ANX4 upregulation	Unknown	Paclitaxel resistance
UGT1A1 upregulation	Detoxification of SN-38	Topoisomerase I inhibitor resistance
ABCF2 upregulation	Unknown	Platinum resistance
Altered BAX/Bcl-2 ratio	Alteration of proapoptotic/ antiapoptotic protein ratio mediating apoptotic escape	Tumorigenesis Paclitaxel sensitivity

ANX4, annexin IV; ASC, apoptosis-associated speck-like protein; ERCC1, excision-repair, complementing defective, in Chinese hamster-1; GLRX, glutaredoxin; GPX3, glutathione peroxidase 3; HNF-1β, hepatocyte nuclear factor-1beta; IGF-IR, insulin-derived growth factor-I receptor; Myc, myelocytomatosis viral oncogene homologue; PTEN, phosphatase and tensin homologue; SOD2, superoxide dismutase; TMS, target of methylation-induced silencing; UGT1A1, UDP glycotransferase 1; XPB, xeroderma pigmentosum B.

platinum-based chemotherapy in patients with OCCA. This led the authors to suggest that ABCF2 protein may be a ''prognostic marker'', despite the lack of any survival analysis in the study to merit the claim. In addition, of the 30 OCCA samples analysed, only 20 patients (17 primary and 3 recurrent cases) were used to evaluate the correlation between ABCF2 expression and chemoresponse, with no available data on the type or dose of platinum-based chemotherapeutic regimen used. Hence, whether ABCF2 upregulation translates to a role in drug resistance in OCCA remains questionable.

Recently, a unique 93-gene expression profile, referred to as the chemotherapy response profile (CRP), which is predictive of pathological complete response to first-line platinum/taxane chemotherapy in 60 patients with EOC (of which 92% were of serous histology), has been described.[79] In the analysis, one of the genes associated with chemoresistance was the apoptotic activator BAX, with reduced expression noted in chemoresistant patients. High levels of BAX protein have previously been correlated with sensitivity to paclitaxel and improved survival in patients with EOC.[80] As described earlier, among the immunohistochemical characteristics of OCCA is the notable overexpression of the proapoptotic protein BAX in stage I and II OCCA tumours.[15] In addition, the antiapoptotic protein Bcl-2, which inhibits BAX-mediated apoptosis, has been observed to be more highly expressed in metastatic than in primary OCCA lesions.[81] A p53-mediated pathway has been implicated in the induction of cell death following DNA damage by platinum-based chemotherapeutic agents, which results in a decrease in the relative ratio of Bcl-2/Bax, thus favouring apoptosis.[82] Hence, the presence of a lower relative ratio of Bcl-2/BAX in early-stage OCCA tumours, and a higher relative ratio of Bcl-2/BAX in metastatic OCCA lesions, may account for the dichotomy in outcome observed in good prognosis early-stage OCCA tumours versus the relatively more platinum-resistant and poorer prognosis late-stage OCCA tumours, when compared with their serous counterparts. Table 1 summarises all the molecular characteristics of OCCAs discussed above, and their putative clinicopathological significance.

FUTURE DIRECTIONS

Clearly, OCCA remains a biological and clinical conundrum with numerous issues to be resolved. These include the following:

- What are the prognostic markers in OCCA?
- What is the optimum chemotherapeutic regimen in OCCA? Are OCCA cells platinum-sensitive?
- What are the developmental origins and pathogenic mechanisms of OCCA—can we identify putative oncogenes and tumour suppressor genes, and therefore potential novel therapeutic targets?
- What are the molecular determinants of tumour progression in OCCA?
- Why do stage I OCCA tumours carry a good prognosis equivalent to their serous counterparts, whereas advanced (stage III/IV) OCCA tumours carry a poorer prognosis? Therefore, do the mechanisms driving metastatic behaviour in OCCA contribute to their chemoresistance as well?
- What are the dominant mechanisms of drug resistance in OCCA—can they be reversed? Can these mechanisms be extrapolated to improve our understanding of chemoresistance in non-clear cell subtypes as well?
- Given that both endometrioid EOC with OCCA tumours may arise from endometriosis, do the tumorigenic molecular events in OCCA and endometrioid EOC overlap?
- Is OCCA more closely related in molecular terms to other clear cell tumours, for example, renal clear cell carcinoma, than to other epithelial ovarian cancers? Does the potential exist for crossover therapeutic targets to be developed for clear cell tumours from a variety of tissue types?

The development of high-throughput gene expression and genomic microarray-based analytical platforms are now providing us with a means of answering these important questions by facilitating the characterisation of key genomic and gene-expression changes in OCCA, which may lead to the

Take-home messages

- Ovarian clear cell adenocarcinomas (OCCAs) have a poorer prognosis in advanced stages when compared to other epithelial ovarian carcinoma (EOC) subtypes. Interestingly, this is not the case for early-stage disease.
- Studies suggest that OCCAs are also relatively more chemoresistant compared with other EOC subtypes.
- The mechanisms of tumour development, progression and drug resistance in OCCAs remain largely unknown.
- Further studies using high-resolution microarray technology may improve our understanding of OCCA pathobiology and allow novel therapeutic targets to be identified. In this context, a potential lead is the similarity of the molecular changes noted in OCCA and other clear cell carcinomas, including renal and endometrial clear cell carcinomas.

identification of novel therapeutic targets as well. Unfortunately, the aforementioned studies using these technologies have remained inconclusive, largely due to insufficient sample sizes being analysed. Although no reliable method for formal power calculation has been devised for microarray-based studies, a minimum number of 50 subjects has been suggested.[83] Additionally, as some difficulties may arise in the morphological diagnosis of OCCA—for example, clear cell changes may occur in ovarian serous or endometrioid adenocarcinomas and be mistaken for OCCA—it should be stressed that, in choosing cases for use in these studies, a careful histopathological review should be undertaken by a pathologist with an interest in gynaecological tumours. It is envisaged that with further use of high-resolution array-CGH and gene-expression studies incorporating adequate sample sizes, the enigma of OCCA may finally begin to unravel.

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