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The biology of ovarian cancer: new opportunities for translation

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Abstract

Over the past two decades, the 5-year survival for ovarian cancer patients has substantially improved owing to more effective surgery and treatment with empirically optimized combinations of cytotoxic drugs, but the overall cure rate remains approximately 30%. Many investigators think that further empirical trials using combinations of conventional agents are likely to produce only modest incremental improvements in outcome. Given the heterogeneity of this disease, increases in long-term survival might be achieved by translating recent insights at the molecular and cellular levels to personalize individual strategies for treatment and to optimize early detection.

- Several factors make ovarian cancer a difficult disease to treat effectively. Although many patients experience symptoms, these often overlap with other ailments, and many patients are diagnosed after the cancer has metastasized. Ovarian cancer is also heterogeneous — multiple genetic and epigenetic changes are evident in patients with ovarian cancer; however, how such changes are selected for during tumorigenesis is not yet clear.

- Mutation and loss of *TP53* function is one of the most frequent genetic abnormalities in ovarian cancer and is observed in 60–80% of both sporadic and familial cases. Of the 16 candidate tumour suppressor genes identified to date in ovarian cancer, 3 are imprinted genes. Several growth inhibitory genes are also silenced by methylation or imprinting.

- Inheritance of DNA repair defects contributes to as many as 10–15% of ovarian cancers. The lifetime risk of developing ovarian cancer in mutation carriers varies with the genetic defect (for *BRCA1* 30–60%, for *BRCA2* 15–30% and for hereditary non-polyposis colon cancer 7%).

- At least 15 oncogenes have been implicated in ovarian cancers, and DNA copy number abnormalities have also been found in loci that are known to contain non-coding microRNAs. At least seven signalling pathways are activated in >50% of ovarian cancers, and mutations that affect cell proliferation, apoptosis and autophagy are also evident.

- Ovarian cancer can be split into two groups on the basis of genetic changes: low-grade tumours with mutations in *KRAS, BRAF* and *PIK3CA*, loss of heterozygosity (LOH) on chromosome Xq, microsatellite instability and expression of amphiregulin; and high-grade tumours with aberrations in *TP53* and potential aberrations in *BRCA1* and *BRCA2*, as well as LOH on chromosomes 7q and 9p.

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Competing interests statement
The authors declare no competing financial interests.

- Changes in cell adhesion and motility also contribute to disease development and metastasis. Adhesion of ovarian cancer cells to the mesothelial cells and to the underlying stroma is mediated by CD44, CA125 and b1 intergrin on the surface of ovarian cancer cells that bind to mesothelin and hyaluronic acid on mesothelial cells, or to fibronectin, laminin and type IV collagen in the underlying matrix.

- A crucial goal is to identify patients who would benefit from particular targeted therapies. Given the complexity of crosstalk between protein signalling pathways, predicting the impact and efficacy of any one signalling inhibitor is difficult. Inhibition of multiple pathways will almost certainly be required to substantially affect ovarian cancer growth.

- Effective methods for early detection are needed. Given the prevalence of ovarian cancer, strategies for early detection must have a high sensitivity for early-stage disease (>75%), but an extremely high specificity (99.6%) to attain a positive predictive value of at least 10% (ten operations for each case of ovarian cancer). Using rising values of serum biomarkers such as CA125 to trigger transvaginal sonography is a promising approach.

Ovarian cancer has a distinctive biology and behaviour at the clinical, cellular and molecular levels. Clinically, ovarian cancers often present as a complex cystic mass in the pelvis. Although ovarian cancer has been termed the 'silent killer', more than 80% of patients have symptoms, even when the disease is still limited to the ovaries[1]. These symptoms are, however, shared with many more common gastrointestinal, genitourinary and gynaecological conditions and have not yet proved useful for early diagnosis. Metastases can occur through lymphatics to nodes at the renal hilus or through blood vessels to the parenchyma of the liver or lung. Most frequently, small clusters of cancer cells are shed by the ovary and implant on the peritoneal surface, forming numerous nodules. For cancer in the ovary, unlike cancers at many other sites, no anatomical barrier exists to widespread metastasis throughout the peritoneal cavity. Tumour implants block lymphatic vessels that pass through the diaphragm, preventing the outflow of ascites fluid that leaks from disordered tumour vessels in the presence of high levels of tumour-derived vascular endothelial growth factor A (VEGFA), which is a vascular permeability factor[2]. Antibodies that neutralize VEGFA have decreased the accumulation of ascites in animal models and in clinical studies[3, 4]

Despite ongoing efforts to develop an effective screening strategy (Box 1), only 20% of ovarian cancers are diagnosed while they are still limited to the ovaries (stage 1). At this stage, up to 90% of patients can be cured using currently available therapy[5]. After the disease has metastasized to the pelvic organs (stage 2), the abdomen (stage 3) or beyond the peritoneal cavity (stage 4), the cure rate decreases substantially. Ovarian cancer is one of the few malignancies in which cytoreductive surgery is carried out to remove the bulk of the tumour, even when complete resection is impossible. At least 70% of ovarian cancers will respond to a combination of platinum- and taxane-based chemotherapy administered after surgery. In at least one-half of these patients, residual cancer cannot be detected using imaging studies and serum markers after 5 months of treatment.

Box 1|Ovarian cancer screening

Despite advances in therapy, ovarian cancer remains the most lethal of the gynaecological cancers. This is largely related to late diagnosis122. Although up to 90% of stage I patients with ovarian cancer can be cured with conventional surgery and chemotherapy, only 20% of ovarian cancers are currently detected in stage I owing to the absence of an effective screening strategy. Given its prevalence, strategies for early ovarian cancer detection must have a high sensitivity for early-stage disease (>75%), but an extremely high specificity (99.6%) to attain a positive predictive value of at least 10% (10 operations for each case of

ovarian cancer). Transvaginal sonography (TVS), serum markers and a combination of the two modalities have been evaluated for their ability to detect ovarian cancer at early stages. Among the serum markers, cancer antigen 125 (CA125) has received the most attention, but lacks the sensitivity or specificity to be used alone as a screening test when measured on a single occasion[133]. Greater specificity can be achieved by combining CA125 and TVS or by monitoring increases in CA125 over time. Two-stage screening strategies promise to be cost effective; in these strategies, abnormal serum assay results are used to prompt the application of TVS to detect lesions that require laparotomy. Accrual has been completed for a trial with 200,000 subjects in the United Kingdom that will test the ability of increased CA125 levels to prompt TVS and subsequent exploratory surgery. Data from the first 2 years of the trial suggest that this strategy could increase the percentage of disease detected at early stages (48%) with adequate sensitivity (89.4%) and specificity (99.8%)[121].

As only 80% of ovarian cancers express CA125, additional markers will be required to detect all early-stage disease. New biomarkers have been identified using the empirical generation of monoclonal antibodies, gene expression array analysis, proteomics, lipomics and by cloning related proteases. One of the most promising biomarkers is whey-associated protein four-disulphide core domain protein 2 (WFDC2; also known as HE4), which might be transcriptionally upregulated by nuclear factor-κB[123]. At a tissue level, >95% of ovarian cancers can be identified with panels of four or five biomarkers[124]. Two recent abstracts have reported that, in serum, panels of four conventional or proteomic markers have detected 87–90% of stage I cancers[125, 126]. The development of multiplex technologies that simultaneously measure multiple serum markers and the creation of statistical methods that increase sensitivity without sacrificing specificity are promising.

Strategies for the early detection of ovarian cancer depend on several biological assumptions: first, that most ovarian cancers must be clonal and arise from the ovary or fallopian tube in which TVS can detect early tumour masses; second, that the interval before metastasis must be sufficiently long to allow cost-effective annual screening; and third, that most advanced-stage disease must develop from clinically detectable stage I or II lesions. In the case of sporadic disease, 90% of tumours are clonal, most arise from the ovary or fallopian tube, and estimates from preclinical increases in CA125 and from comparison of prevalent and incident cases in the UKCTOCS (UK Collaborative Trial of Ovarian Cancer Screening) are consistent with a lead time of 1.9–2 years[121]. Moreover, a similar profile of gene expression is observed in early- and late-stage high-grade serous cancers[127]. However, these assumptions might not apply to familial ovarian cancer, which is often multifocal, can show *TP53* mutations and metastatic potential in cysts of <1 cm and, at least anecdotally, can present within 3 months of a normal TVS and CA125 result[122]. Although patients with *BRCA1* or *BRCA2* mutations are arbitrarily screened at 3–6 month intervals, the prophylactic removal of both ovaries and fallopian tubes (salpingo-oophorectomy) is generally recommended as soon as women at risk have completed their families.

Small numbers of drug-resistant cells can, however, persist for many months and remain dormant in the peritoneal cavity, only to grow progressively, leading to the death of the patient despite aggressive treatment of recurrent disease. Metastatic nodules form fibrous adhesions between loops of the bowel, causing intestinal obstruction that prevents normal alimentation, leading to malnutrition and eventual death from factors that include intercurrent infection. Given the importance of disease on the peritoneal surface, intraperitoneal delivery of chemotherapy to achieve high local concentrations of a drug has substantially improved the survival of patients who have minimal gross disease remaining after surgery and who can tolerate the side effects of treatment[6]. Thus, the clinical biology of ovarian cancer suggests that late diagnosis and the persistence of dormant, drug-resistant cancer cells limit our ability to cure this disease.

At the cellular and molecular levels, ovarian cancers are remarkably heterogeneous. The normal ovary is a complex tissue with several distinct components. Although ovarian cancers can develop from germ cells or granulosa–theca cells, more than 90% of ovarian cancers have an epithelial histology and are thought to arise from cells that cover the ovarian surface or that line subsurface inclusion cysts[7] Cancers that have a similar histology can also arise from the lining of the fallopian tube, deposits of endometriosis or the surface of the peritoneal cavity. Substantial heterogeneity has been observed in the cellular grade, proliferative index and histotype of ovarian cancers (Box 2).

Box 2|Histotypes of epithelial ovarian cancer

Unlike most cancers, which become less differentiated during transformation, epithelial ovarian cancers develop from simple flattened epithelial cells into four distinct main histotypes (see the figure) that resemble the well-differentiated normal cells that line the fallopian tube (serous), endometrium (endometrioid) and endocervix (mucinous), or that form nests within the vagina (clear cell). At a molecular level, altered patterns of gene expression in different histotypes have correlated with distinctive patterns of gene expression in the normal fallopian tube, endometrial and intestinal mucosa[128]. Histotypes have also correlated with the abnormal re-expression of homeobox (Hox) genes that are normally only expressed during gynaecological organogenesis129. *HOXA9, HOXA10, HOXA11* and *HOXA13* are associated with the developing fallopian tube, uterus, lower uterine segment and cervix, and upper vagina, respectively. Although these genes are not expressed in the normal ovarian surface epithelium, *HOXA9* is expressed in serous, *HOXA10* in endometrioid and *HOXA11* in mucinous ovarian cancers. Moreover, forced expression of each gene in spontaneously transformed undifferentiated murine ovarian surface epithelium cells can induce the formation of histotype-specific cancers. The distinct ovarian cancer histotypes differ with regard to their epidemiology, genetic changes, gene expression, tumour markers and responsiveness to therapy. Although each histotype shows a distinctive pattern of gene expression, similarities have been observed between serous and endometrioid subtypes in some studies[130, 131] and between endometrioid and clear-cell histology in others[126]. Clear-cell and mucinous cancers generally do not respond as well as serous and endometrioid cancers to platinum- and taxane-based chemotherapeutic regimens.
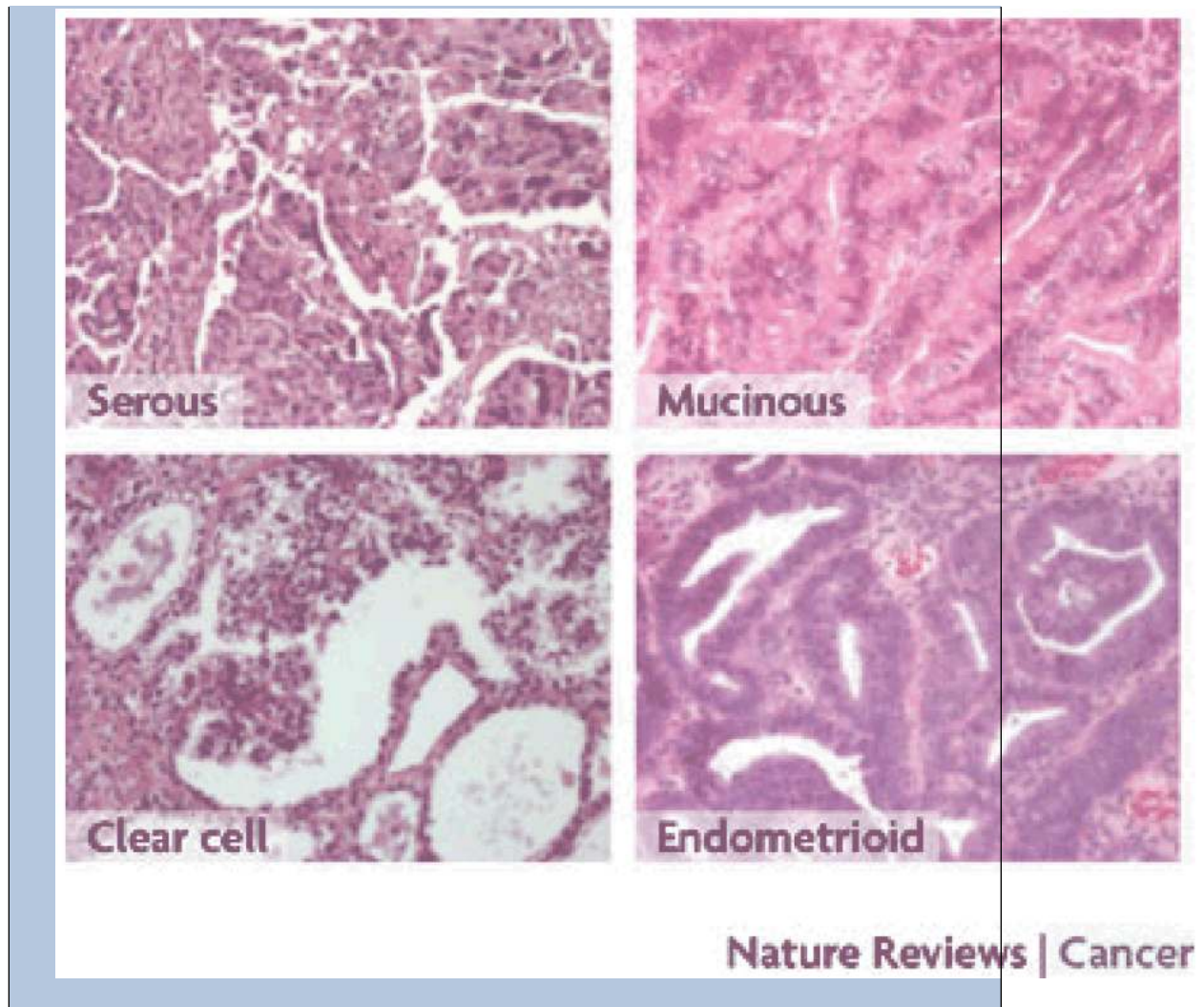
In addition to invasive low-grade and high-grade ovarian cancers, borderline tumours of low malignant potential (LMP) show the epithelial proliferation associated with malignancy without obvious invasion of the underlying stroma. In approximately 10% of cases, borderline cancers can metastasize, recur only after many years and eventually prove lethal. In contrast to high-grade disease, low-grade ovarian cancers and LMP tumours generally resist conventional cytotoxic chemotherapy.

The phenotype of the ovarian cancer stem cell has not been completely defined. Side populations of murine ovarian cancer cells that exclude Hoechst dyes during cell sorting show increased tumour-initiating activity *in vivo*[78]. When human ovarian cancer cells are dissociated from surgical specimens, as few as 100 CD44$^+$CD117$^+$ cells can establish growth as xenografts, whereas 10^5 CD44$^-$CD117$^-$ cells cannot establish growth, suggesting that CD44$^+$CD117$^+$ cells are cancer stem cells[8]. Tumour-initiating cells show increased chemoresistance to cisplatin or paclitaxel and upregulation of stem cell markers (BMI1, stem cell factor (SCF), NOTCH1, NANOG, nestin, ATP-binding cassette G2 (ABCG2) andOCT4) compared with parental tumour cells grown under similar conditions. In other studies, CD44$^+$MYD88$^+$ ovarian cancer cells showed increased tumour-initiating activity, constitutive nuclear factor- κB (NF-κB) expression, cytokine and chemokine production, high capacity for repair of DNA damage, chemoresistance to conventional chemotherapies and resistance to tumour necrosis factor-α

(TNF α)-mediated apoptosis[9] The relevance of these studies to the heterogeneity of ovarian cancer has yet to be established.

As in other solid tumours, 90% of epithelial ovarian cancers are clonal[10]. They develop from the progeny of single cells that have accumulated a series of 5 or more genetic alterations selected from a repertoire of more than 30 oncogenes and tumour suppressor genes that have been implicated in ovarian oncogenesis. Genetic changes drive altered signalling that induces proliferation, inhibits apoptosis, blocks anoikis, increases motility, adhesion and invasion, and attracts stromal components, including mesenchymal stem cells and new blood vessels. Although many of these changes are observed in cancers at different sites, only those that are distinctive or characteristic of ovarian cancer will be considered in this Review.

Pathogenesis of ovarian cancer

Multiple genetic and epigenetic abnormalities have been detected in ovarian cancers from different individuals[11] (Table 1). Activation of genes occurs through amplification, mutation and hypomethylation, whereas genetic inactivation results from deletion of large chromosomal regions, more limited loss of heterozygosity (LOH) at particular loci and promoter methylation. Evidence for genetic instability is provided by the observation of multiple changes using comparative genomic hybridization, particularly in cancers that have a high histological grade and are of a late stage[12] Only 10–15% of ovarian cancers are associated with germline mutations of *BRCA1, BRCA2* or the heritable non-polyposis colorectal cancer mismatch repair genes, loss of which predisposes to transformation and genetic instability[13].

The source of much genetic damage remains uncertain. Chemical carcinogens with mutagenic potential have generally not been linked to ovarian cancer risk, although exposure to the chronic inflammation produced by talc has been cited as a risk factor in some studies[14, 15]. Consistent with an absence of strong exogenous chemical carcinogens, the pattern of *TP53* mutations in sporadic ovarian cancers is most consistent with spontaneous mutations that occur during cell proliferation[16]. Epithelial cells covering the ovary are generally quiescent and have a low proliferative index. Following the rupture of mature follicles to release oocytes, epithelial cells proliferate to repair damage to the ovarian surface. Ovulation is also crucial to the development of epithelial cell-lined subsurface inclusion cysts from which a substantial fraction of ovarian cancers develop. Epidemiological factors that increase the number of ovulatory cycles — early onset of menses, late menopause and infertility — increase the risk of ovarian cancer, whereas factors that decrease ovulation — multiple pregnancies, prolonged lactation and use of oral contraceptives — decrease the risk of ovarian cancer in later life. After menopause, proliferation of ovarian epithelial cells could be driven by increased levels of follicle-stimulating hormone, luteinizing hormone, oestrone and androgen. Given the low rates of spontaneous mutation, it is not surprising that few oncogenes are activated by mutation and several of the most frequently affected tumour suppressor genes are either haploinsufficient or are imprinted genes and are therefore heterozygous and subject to LOH.

Tumour suppressor genes

Many well-established and putative tumour suppressorgenes have been implicated in ovarian oncogenesis (Table 2)[11]. Although oncogenes can be activated by a single genetic event, both alleles of a tumour suppressor gene generally must be inactivated to lose function. If spontaneous mutation during proliferation is an important mechanism in ovarian oncogenesis, loss of tumour suppressor function could be less frequent than in carcinogen-driven cancers with higher rates of mutation. There are, however, several important exceptions to the generalization that two genetic events must occur to lose tumour suppressor function.

TP53

Owing to the dominant negative activity of mutant p53 proteins, *TP53* function can be lost with a single genetic event. Mutation and loss of *TP53* function is one of the most frequent genetic abnormalities in ovarian cancer and is observed in 60–80% of both sporadic and familial cases. *TP53* mutation and consequent overexpression is seen in approximately 4% of pre-invasive borderline tumours[17], 10–20% of early cancers and 40–60% of advanced cancers, and correlates with metastatic potential[18, 19]. Mutated *TP53* has been found in microscopic ovarian cancers in oophorectomy specimens resected prophylactically from women who carry *BRCA1* or *BRCA2* mutations, which is consistent with an early role in familial disease. *TP53* mutation has been associated with resistance to platinum-based therapy in some studies. Although 43% of 215 published analyses from 64 papers reported a correlation between *TP53* mutation and a clinical end point that was relevant to chemoresistance, none of the 6 most crucial studies showed a statistically significant correlation[20]. Interestingly, *TP53* mutations have been associated with a short-term survival benefit[19].

Clinically, restoration of p53 function has been attempted using a replication-deficient adenoviral vector containing recombinant wild-type *TP53* that was delivered into the peritoneal cavities of heavily pretreated patients with or without additional chemotherapy. Despite a decrease in the levels of the cancer antigen 125 (CA125) serum biomarker in half of the evaluable patients, this approach was abandoned owing to the failure of p53 therapy to provide a more dramatic improvement in the response to retreatment with carboplatin and paclitaxel[21]. The selective replication of a genetically modified E1B-deficient adenovirus (ONYX-015) has been achieved, allowing the lysis of ovarian cancer cells with *TP53* mutations in culture and the inhibition of xenograft growth while sparing normal cells. Intraperitoneal administration of ONYX-015 in the clinic failed, however, to inhibit ovarian cancer growth[22]. *MDM2* is an E3 ubiquitin ligase that binds p53 and targets it for proteasomal degradation. In leukaemias with wild-type p53, disruption of the p53–MDM2 association with small molecular mass compounds such as nutlins increased p53 stability and induced apoptosis[23]. Approximately one-third of ovarian cancers overexpress MDM2 and a similar strategy might be pursued in those cancers with high levels of MDM2 and low levels of wild-type p53.

PTEN

Aside from *TP53*, inactivating somatic mutations of other growth suppressive genes are uncommon in sporadic epithelial ovarian cancer. Inactivating mutations of *PTEN*, for example, are found in only 3–8% of sporadic cancers, which are largely of the endometrioid histotype and are usually of a low grade.

Imprinted tumour suppressor genes

The function of imprinted tumour suppressor genes can also be lost in a single step. Approximately 70 human genes are known to be imprinted: only one allele is expressed in each cell of the embryo and adult and silencing of the maternal or paternal allele is inherited despite a normal nucleotide sequence. Of the 16 candidate tumour suppressor genes identified to date in ovarian cancer, 3 are known to be imprinted: *ARHI* (also known as *DIRAS3*), pleiomorphic adenoma gene-like 1 (*PLAGL1*, also known as *LOT1*) and paternally expressed 3 (*PEG3*). All three genes are downregulated in a substantial fraction of ovarian cancers.

ARHI encodes a 26 kDa GTPase that shares homology with Ras, but has a distinctive 34 amino acid amino-terminal extension that mediates its tumour-suppressive effect. The functional allele of *ARHI* is downregulated in >60% of ovarian cancers owing to LOH (30%), promoter hypermethylation (10%), transcriptional regulation (>20%) or decreased mRNA half-life[24].

Expression of *ARHI* is associated with prolonged progression-free survival. Re-expression of *ARHI* inhibits proliferation, motility and angiogenesis, and induces autophagy (see below). *PLAGL1* encodes a 55 kDa zinc-finger protein that is downregulated in 39% of ovarian cancers through LOH and transcriptional regulation[25]. Re-expression of *PLAGL1* inhibits proliferation in culture and tumour formation in xenografts. *PEG3* encodes a 140 kDa Krüppel-type zinc-finger protein that is downregulated in 75% of ovarian cancers by LOH (20%), promoter methylation (26%) and, in all probability, transcriptional regulation[26]. Re-expression of *PEG3* inhibits growth and induces apoptosis through translocation of BAX downstream of p53. However, the persistence of *PEG3* expression does not improve prognosis. Treatment with demethylating agents and histone deacetylase inhibitors has induced expression of *ARHI* and *PEG3* in ovarian cancer cell lines after treatment, correlating with growth inhibition[27]. Consequently, increased expression of imprinted and silenced genes might prove useful as a biomarker for monitoring epigenetic therapy.

Other epigenetically silenced genes

Several growth inhibitory genes are silenced by methylation or imprinting in ovarian cancer including *RASSF1*, deleted in lung and esophageal cancer 1 (*DLEC1*) and opioid binding protein/cell adhesion molecule-like (*OPCML*) (Table 2). Additional growth-inhibitory genes such as *DLEC1* are directly or indirectly downregulated by histone deacetylation. A recently published abstract showed that the histone deacetylase inhibitor belinostat has clinical activity against borderline ovarian cancers that are generally refractory to chemotherapy[28]. Platinum resistance has been associated with CpG methylation of the *MLH1* mismatch repair gene in relapsed invasive ovarian cancers and can be reversed with demethylating agents in preclinical models[29]. An initial report on a Phase II trial at M. D. Anderson Cancer Center showed that the combination of the demethylating agent azacytidine and carboplatin had clinical activity in 22% of 18 patients with platinum-resistant ovarian cancer[30].

BRCA1 and BRCA2

Inheritance of DNA repair defects contributes to as many as 10–15% of ovarian cancers[31,32]. The lifetime risk of developing ovarian cancer in mutation carriers varies with the genetic defect (for *BRCA1* 30–60%, for *BRCA2* 15–30% and for hereditary non-polyposis colon cancer 7%). In contrast to sporadic disease, BRCA-related familial ovarian cancers are more frequently multifocal, with genetically distinct clones involving multiple sites[33], and progress faster, but are more sensitive to platinum-based chemotherapy[34]. BRCA1 and BRCA2 are required for reliable DNA double strand break repair by homologous recombination[35, 36]. As in familial breast cancers, an inactivated allele is inherited by all cells, and loss of *BRCA1* or *BRCA2* function occurs in ovarian cancer cells through LOH of the normal allele. Cells lacking BRCA1 or BRCA2 repair DNA by error-prone mechanisms such as non-homologous end joining, leading to chromosomal rearrangements and genomic instability. Ovarian cancer cells with truncating mutations of *BRCA1* or *BRCA2* or with lower expression of these genes are less able to repair DNA damage induced by platinum compounds. Indeed, platinum chemoresistance can arise from mutations that restore the *BRCA2* open reading frame and thus the cellular capacity for homologous recombination[37,38]. Similar to platinum drugs, poly (ADP-ribose) polymerase (PARP) inhibitors are particularly effective in the presence of defects in homologous recombination repair, and clinical trials with these agents are underway in ovarian cancer patients with inherited *BRCA1* and *BRCA2* mutations[39, 40].

Although the *BRCA1* and *BRCA2* genes have been thought to be mutated only rarely in sporadic ovarian cancers, somatic mutations have recently been documented in approximately 10% of non-familial cases[41], and expression of *BRCA1* and *BRCA2* can also be silenced by methylation in additional cancers[42]. Remarkably, the gene expression profiles in sporadic cases have been

reported to fall into two categories that resemble the expression in cancers from patients carrying either *BRCA1* or *BRCA2* mutations, although this observation requires confirmation[43]. High-grade sporadic serous epithelial ovarian cancer might show dysfunction of the BRCA1 or BRCA2 pathway, contributing to sensitivity to platinum-containingtherapies. PARP inhibitors might also find broader clinical application in sporadic cancers in which these pathways are also defective.

Oncogenes

At least 15 oncogenes have been implicated in ovarian cancers (Table 3)[11], and 11 of these oncogenes show genomic amplification[11, 44]. DNA copy number abnormalities have also been found in 37% of 283 loci that are known to contain non-coding microRNAs[45]. In contrast to copy number anomalies, activating mutations in oncogenes are uncommon in the most frequently occurringhistotypes.

KRAS is mutated in >20% of the less common low-grade (type I) cancers, but *KRAS*mutations are rare in the more numerous high-grade (type II) lesions[132] (Box 3). PI3K catalytic subunit-α (*PIK3CA*) mutations occur commonly only in the small subset of endometrioid and clear-cell ovarian cancers. Thus, ovarian cancer can be split into two groups: low-grade tumours with mutations in *KRAS*, *BRAF* and *PIK3CA*, LOH on Xq, microsatellite instability[46] and expression of amphiregulin; and high-grade tumours with aberrations in *TP53* and potential aberrations in *BRCA1* and *BRCA2*, as well as LOH on 7q and 9p[47, 48].

Box 3|Molecular classification of epithelial ovarian cancers

Type I ovarian cancers constitute a minority of epithelial lesions and include low-grade and borderline serous cancers, endometrioid, mucinous and clear-cell cancers[132]. This group has more frequent *PTEN*, PI3K catalytic subunit-α (*PIK3CA*), *KRAS, BRAF* and b-catenin (*CTNNB1*) mutations, along with genomic stability and *TP53*mutations in only a small fraction of cases. Type II ovarian cancers constitute most epithelial ovarian cancers and include high-grade serous cancers, mixed malignant mesodermal tumours, carcinosarcomas and undifferentiated cancers[132]. These tumours have *TP53* mutations in up to 80% of cases, along with marked genomic instability. Type II cancers arise more frequently from the fallopian tubes and the peritoneum. Ovarian cancers that arise in women with inherited *BRCA1* and *BRCA2*mutations are usually type II tumours.

Type	Histology
I	Low-grade serous carcinoma
I	Low-grade endometrioid carcinoma
I	Mucinous carcinoma
I	Clear cell carcinoma
II	High-grade serous carcinoma
II	High-grade endometrioid carcinoma

*
Endometriosis and adjacent low-grade endometrioid carcinoma share common genetic events such as loss of heterozygosity at the same loci involving the same allele (for example, *PTEN*). By contrast, high-grade and poorly differentiated endometrioid carcinomas are similar to high-grade serous carcinomas.

‡*PIK3CA* at 3q26 encodes the p110α catalytic subunit of PI3K[19].

Well-differentiated tumours with a low histological grade are associated with a better prognosis than are high-grade neoplasms, despite the resistance of low histological grade tumours to chemotherapy. At least a fraction of low-grade serous cancers seem to arise from LMP tumours. Karyotypically, LMP tumours are generally diploid[49] and have few DNA copy number changes. Expression of the oestrogen receptor is found more frequently in LMP and low-grade ovarian cancers, suggesting that hormonal manipulation might be effective for controlling the growth of these neoplasms[50]. Ovarian surface epithelial cells immortalized with human telomerase reverse transcriptase and SV40 t antigen have been transformed with mutant *HRAS* or *KRAS*, and lead to cells that grow slowly but progressively as xenografts with serous papillary histology in the peritoneal cavity of nude mice[51]. Gene expression profile analysis of the transformed cells showed the increased expression of several cytokines, including interleukin 1 (IL-1), IL-6 and IL-8, which are upregulated by the NF-κB pathway[51]. Each of these molecules might provide targets for therapeutic intervention in type I cancers with Ras mutations.

The small G-protein RAB25 is amplified and upregulated in most ovarian cancers and regulates motility, aggressiveness, apoptosis and autophagy[52]. RAB25 also mediates survival in response to stress, such as that induced by chemotherapy, ultraviolet radiation, serum depletion and glucose starvation. Delivery of specific small-interfering RNAs (siRNAs) could allow targeting of RAB25.

Several other amplified oncogenes are potential targets for ovarian cancer therapy. Small molecule inhibitors of Aurora kinases are currently being evaluated in several cancers[53]. Antibodies that inhibit NOTCH3 or its ligands are being developed[54]. New gold compounds

have been shown to target the N-terminal PHOX-BEM1 domain of PKC ι that is required for PKC ι-driven transformation.

Signalling

At least seven signalling pathways are activated in >50% of ovarian cancers (Box 4).

Box 4|Development of targeted therapies

New screening small-interfering (siRNA) libraries have been used to identify targets such as Src and Akt that increase the cytotoxicity of paclitaxel in ovarian cancer cells. Phase II clinical trials of the Src inhibitor dasatinib have been initiated.

Although small molecule inhibitors can be developed for intracellular targets and antibodies can be developed for the inhibition of cell surface receptors, growth factors and cytokines, the therapeutic use of siRNA promises to provide more rapid translation of this approach to the clinical setting. Recent studies have shown that the intravenous injection of siRNA in neutral liposomes can deliver adequate amounts of siRNA to inhibit the growth of intraperitoneal xenografts[104].

New technologies are being introduced that allow the simultaneous measurement of multiple signalling pathways. Gene expression analysis has identified ovarian cancer signatures that predict optimal cytoreductive surgery, poor overall survival and chemoresistance, although the predictive values of these signatures have not been sufficient to affect patient management. Recently, gene expression profiles have also been reported that identify the activation of particular signalling pathways (see the table) and responsiveness to targeted therapies. New reverse-phase protein lysate arrays can measure the simultaneous activation of multiple signalling pathways, thus allowing a more accurate definition of signalling heterogeneity and prediction of responsiveness to targeted therapies.

Signalling pathway	Percentage of cancers in which activation is observed
PI3K	70%
Src	>50%
IL-6–IL-6R; Jak–STAT3	70%
LPA	90%
MEKK3–IKK–NK-κB	>50%
Mullerian inhibitory substance receptor	>50%
PKCι	78%
Ras–Mek–Mapk[*]	<50% (activated in most low-grade type I cancers)

[*] As Ras mutations are only common in low-grade ovarian cancers, Ras and Mek inhibitors are likely to be most efficacious in low-grade tumours, IKK, inhibitor of nuclear factor-κB kinase; IL-6, interleukin 6; IL-6R, IL-6 receptor; Jak, Janus kinase; LPA, lysophosphatidic acid; NK-κB, nuclear factor-κB; PKCι, protein kinase Cι; STAT3, signal transducer and activator of transcription 3.

Epidermal growth factor receptor (EGFR) mutations are rare in ovarian cancer, and the receptor is not markedly overexpressed. Gefitinib and erlotinib, which are inhibitors of EGFR, stabilized disease in 11–44% of patients with ovarian cancer but produced objective regression in only 4–6% of cases[55, 56]. As shown in colorectal cancers, activation of the Ras–Mapk signalling pathway might also minimize the effect of EGFR inhibitors[57]. *ERBB2* (also known as *HER2*) is not frequently amplified and is overexpressed in only 11% of ovarian cancers[58]. Objective responses were observed in only 7% of 41 patients with ovarian cancer that overexpressed *ERBB2* who were treated with trastuzumab, and only 1 complete response and 2 partial responses were observed[58].

The PI3K pathway is activated in approximately 70% of ovarian cancers, and activation of this pathway is associated with resistance to cytotoxic chemotherapy. Activation of the PI3K pathway can be driven by gene amplification (*PIK3CA* and *AKT2*), activating mutations (*PIK3CA*) or inactivating mutations (*PTEN*), but in many cancers the pathway is activated by autocrine or paracrine signalling through protein tyrosine kinase growth factor receptors. Inhibitors of PI3K and Akt prevent the growth of ovarian cancer xenografts and potentiate the cytotoxic effects of paclitaxel and cisplatin[59]. Perifosine inhibits Akt, and an ovarian cancer clinical trial is currently underway at M. D. Anderson Cancer Center combining perifosine with docetaxel. More specific Akt inhibitors are being developed and PI3K inhibitors are entering Phase I–II trials[60].

IL-6 is overexpressed in most ovarian cancers, providing autocrine stimulation of the IL-6 receptor, which activates Janus kinase 2 (JAK2), facilitating phosphorylation and nuclear translocation of the signal transducer and activator of transcription 3 (STAT3), which upregulates genes that stimulate proliferation, inhibit apoptosis and induce angiogenesis. Nuclear localization of activated phosphorylated STAT3 occurs in more than 70% of ovarian cancers and is associated with decreased overall survival[61]. Activated STAT3 also translocates

to focal adhesion complexes and stimulates motility in combination with SRC. In addition to antibodies against IL-6 and inhibitors of JAK2 (Refs 62, 63), new inhibitors of STAT3 are being developed that might be used to target most ovarian cancers[64].

Lysophosphatidic acid (LPA) is produced by the phosphodiesterase autotoxin (ATX; also known as lysophospholipase D). The G protein-linked LPA receptors LPAR2 and LPAR3 are upregulated during the malignant transformation of ovarian surface epithelial cells. LPAR3 responds to LPAs with unsaturated fatty acyl chains that are produced by ovarian cancers. Interestingly, cyclic phosphatidic acid blocks ATX, reducing LPA levels and metastasis, but not the growth of primary cancers[65]. LPA-neutralizing antibodies have been developed, and LPA receptor inhibitors are being sought that might block the proliferation of ovarian cancer cells[66].

The NF-κB transcription factor is constitutively activated in more than half of ovarian cancers[67, 68] through signalling initiated by several cytokines (IL-1 and TNFα) and growth factors (EGF). Inactive NF-κB is complexed with inhibitor of NF κB (IκB) and is generally activated by the IκB kinase (IKK) complex, which contains two kinase subunits (IKKα and IKKβ) and a regulatory subunit (IKKγ)[69]. MEKK3 is one of several kinases that can activate the IKK complex[70, 71] and is overexpressed in >50% of ovarian cancers. The resultant activation of NF-κB upregulates anti-apoptotic genes (for example, *CFLAR*), antioxidant proteins (superoxide dismutase or ferritin heavy chain), growth regulatory cytokines (IL-6 or growth regulated-α (GRO1)) and angiogenic factors (IL-8)[71]. Selective inhibition of NF-κB has been difficult to achieve, but gene therapy with adenoviral E1A reduces NF-κB signalling and increases sensitivity to paclitaxel in xenograft models, which has prompted a clinical trial at the University of Texas M. D. Anderson Cancer Center of liposomal E1A in combination with paclitaxel in patients with intra-abdominal recurrence of ovarian cancer.

Tumour biology

Proliferation

The fraction of cycling cells in different ovarian cancers varies over a wide range from 1% to 79% (Ref. 72). Upregulation of cyclin D1 or E1, E2F1 or cyclin-dependent kinases (CDK2), and downregulation of CDK inhibitors (p16, p21 and p27) have been observed in a fraction of ovarian cancers, and the levels of cyclins and CDKs correlate with prognosis[73, 74]. In addition to intrinsic deregulation of checkpoints in the cell cycle, a range of autocrine and paracrine growth factors stimulate ovarian cancer proliferation, including EGF, transforming growth factor-α(TGFα), amphiregulin, heregulin, VEGFA, insulin-like growth factor 1 (IGF1), IL-6 and LPA.

In normal ovarian surface epithelial cells, autocrine growth inhibition is maintained by TGFβ[75]. In approximately 40% of ovarian cancers, expression of or responsiveness to TGFβ is lost. Although mutation of SMAD4 is sometimes observed, TGF βRI and TGF βRII receptors are generally intact, as is Smad signalling downstream of the receptors. Loss of growth inhibition and increased invasiveness might relate to ecotropic viral integration site 1 (*EVI1*) overexpression in 43% of ovarian cancers[76]. *EVI1* inhibits transcription of TGFβ-responsive genes. Ski-like protein (SKIL) and runt-related transcription factor 1 (RUNX1), which bind to and regulate Smads, are also deregulated in ovarian cancer.

Mullerian inhibition substance (MIS) is a homologue of TGFβ and binds to a TGFβ-like receptor, leading to regression of gynaecological precursors during the development of normal male embryos[77]. MIS also inhibits the growth of epithelial ovarian cancer cells by binding to MISII G protein-coupled receptors and upregulating p16, leading to cell cycle arrest in G1. MISII receptors have been detected in 56% of human ovarian cancers, and clonogenic growth

can be inhibited with MIS in more than 80% of the cancers that express MISII receptors[78]. Recombinant MIS also increases the activity of cisplatin against ovarian cancer cell lines[79]. As sufficient quantities of MIS become available, clinical trials could be undertaken.

Apoptosis

Decreased sensitivity to apoptotic stimuli occurs in epithelial ovarian cancer. The pro-apoptotic receptor CD95 was detected in 85% of inclusion cysts, 94% of cystadenomas, 35% of borderline tumours and 4% of invasive cancers[80]. Thus, invasive ovarian cancers are likely to resist the apoptosis that is induced by exposure to CD95 ligand or agonistic anti-CD95 antibodies. When compared with normal ovaries, overexpression (4–10-fold) of the anti-apoptotic proteins BCL-2, BCL-X_L (also known as BCL2L1) and survivin (also known as BIRC5) was observed in >70% of invasive epithelial ovarian cancers[81]. The altered expression of apoptosis-regulating proteins has also been associated with poor outcomes (decreased BAX), platinum resistance (XIAP upregulation) and taxane resistance (BCL-2 and survivin overexpression)[82, 83, 84].

Autophagy

During autophagy, cellular organelles are enclosed and digested in double-walled autophagosomes, recycling amino acids and fatty acids and providing substrates for the generation of ATP. Autophagy can serve as a short-term survival mechanism that sustains cancer cells in an environment that is poor in nutrients, but prolonged autophagy can induce nonapoptotic cancer cell death. The development of breast cancers in beclin^{+/−} mice with impaired autophagy supports the notion that autophagy can suppress cancer progression[85]. However, recent studies suggest that autophagy might be crucial for the maintenance of tumour dormancy.

Expression of the imprinted tumour suppressor gene *ARHI* is decreased or lost in 60% of ovarian cancers. Re-expression of *ARHI* at physiological levels blocks growth, motility and angiogenesis, and also induces autophagy by inhibiting PI3K and downregulating mTOR activity[86]. In addition to triggering autophagy, ARHI participates directly in autophagosome formation by upregulating the ATG4 enzyme that processes the microtubule-associated protein LC3I to LC3II. Although *ARHI* re-expression caused autophagic death of ovarian cancer cells in culture within days, induction of *ARHI* in xenografts does not kill ovarian cancer cells, but instead induces tumour dormancy for a period of weeks[86]. Ovarian cancer xenografts rapidly resume growth when *ARHI* is downregulated. Treatment with chloroquine, an inhibitor of autophagy, substantially delays the outgrowth of xenografts, which is consistent with the possibility that autophagy is required for tumour cell survival. Cytokines (IGF1, IL-8 and VEGFA) and matrix proteins (fibronectin and collagen) that are found in the microenvironment of xenografts can rescue ovarian cancer cells from autophagic death in culture and reverse ARHI-induced inhibition of PI3K signalling. If autophagy is required for tumour dormancy and if this model is relevant to human ovarian cancer, strategies could be devised using siRNAs (to target beclin and ATG5) and small molecules (class III PI3K inhibitors) to prevent autophagy in dormant cancer cells. Alternatively, crucial growth factors (IL-8 and VEGFA) that sustain autophagic cancer cells or their receptors (IGF1R and VEGFR) could be targeted using antibodies or protein tyrosine kinase inhibitors.

Motility and invasion

To metastasize, ovarian cancer cells must not only detach from neighbouring epithelial cells and adopt a mesenchymal phenotype, but they must also acquire the ability to move and invade stroma, vessels and the walls of the cysts in which they arise. Signals regulating motility and invasion are transduced through the Ras–Mapk, PI3K–Akt–p70S6K and STAT3 signalling pathways. A range of ligands activate these pathways, including VEGFA, EGF, heregulin,

hepatocyte growth factor, macrophage colony-stimulating factor, TGFβ, bone morphogenetic protein 4, TNFα and LPA[87, 88]. LPA is frequently detectable in serum and ascites, and also induces the secretion of VEGFA, IL-6, IL-8 and GRO1 by ovarian cancer cells[89]. Interestingly, stress hormones, including adrenaline, noradrenaline and cortisol, have also recently been shown to upregulate matrix metalloproteinase 2 (MMP2) and MMP9 in ovarian cancer cells, thereby increasing invasion[90]. These experiments have provided one of the first physiological mechanisms by which psychological stress might influence tumour progression.

Directed motion of cancer cells and invasion can also be stimulated by the binding of cancer-associated integrins to the extracellular matrix. Clustering of collagen-binding integrins during the adhesion of ovarian cancer cells to collagen activates the Src family kinases to induce expression of early growth response 1 (*EGR1*), resulting in transcriptional activation of the *MMP14* promoter and subsequent collagen invasion catalysed by MMP14 (Ref. 91). Laminin, collagen and fibronectin can all increase chemotactic activity, whereas increased invasion is observed only with laminin and collagen. α3, α6 and β1 integrin-mediated signalling through Ras–Mapk, Erk and Akt regulate both processes.

Effector proteases, which are crucial for invasion, have been detected in ovarian cancer cells and in stromal components. MMP2, MMP7, MMP9, urokinase plasminogen activator and the kallikreins are found in ovarian cancer cells in culture and in surgical specimens[92]. Twelve of the fifteen human kallikreins are transcriptionally upregulated in ovarian cancer. Kallikreins degrade matrix components, including fibronectin, vitronectin, laminin and collagens I, II, III and IV, thereby increasing ovarian cancer cell invasiveness[93]. They are therefore being evaluated as biomarkers for early detection or prognosis in ovarian cancer[94].

Adhesion

Metastasis to the peritoneal surface requires the adhesion of ovarian cancer cells to the mesothelial cells that line the cavity and to the underlying stroma. Adhesion is mediated by β integrins, CD44 and CA125 (Ref. 95) on the surface of ovarian cancer cells that bind to mesothelin and hyaluronic acid on mesothelial cells, or to fibronectin, laminin and type IV collagen in the underlying matrix. CA125 is a high molecular mass (1 MDa) glycosylated transmembrane mucin that is expressed by 80% of ovarian cancers[95]. The N-glycans of CA125 bind to mesothelin and might be the initial point of contact between ovarian cancer cells and the peritoneal surface[96]. Mesothelin-specific antibodies block the adhesion of ovarian cancer cells to mesothelial cells[97]. In addition, knockdown of CA125 decreases the invasion of ovarian cancers, consistent with active signalling by the intracellular domain of the mucin. Both CA125 and mesothelin are shed into body fluids and are being evaluated as serum biomarkers for the monitoring and early diagnosis of ovarian cancer (Box 1). CD44–hyaluronic acid and β1 integrin–fibronectin, laminin and type IV collagen interactions might also contribute to peritoneal metastases. More than 70% of ovarian cancers show a diverse mixture of CD44 splice variants that can bind to hyaluronic acid[98]. CD44-specific antibodies can partially block adhesion of ovarian cancer cells to peritoneal mesothelial cells[99]. Thus, motility, adhesion and invasion might be inhibited using antibodies (LPA-, CA125-, CD44- and mesothelin-specific) and small molecule inhibitors (adrenergic receptors and proteases). Even after diagnosis and primary treatment, continued metastasis in the peritoneal cavity occurs during the progression of drug-resistant disease, ultimately producing intestinal obstruction. However, given dramatic differences in the rate of intra-abdominal progression from patient to patient, as well as the availability of multiple conventional drugs to treat recurrent disease, the design of appropriate clinical trials to test these strategies is problematic.

Angiogenesis

As is the case in other cancers, tumour vessels must be recruited if ovarian cancers and their metastases are to grow to more than 2 mm^3. Microvessel density correlates with the propensity of ovarian cancers to metastasize and with disease-free survival[100]. VEGFA, LPA, IL-6, IL-8, fibroblast growth factor 2 (FGF2) and FGF1 all contribute to angiogenesis in ovarian cancer[100, 101]. Treatment with bevacizumab, a VEGFA-specific antibody, induced an objective response rate in 16% of patients with recurrent ovarian cancer and stabilized disease for 5.5 months in 50% of patients[102]. In general, anti-angiogenic therapy has been well tolerated, although hypertension has been observed in approximately one-third of the women treated and bowel perforation has occurred in approximately 5% of cases. In combination with cytotoxic chemotherapy, improved response rates have been reported in platinum-resistant disease. Daily metronomic chemotherapy with agents such as cyclophosphamide can take advantage of the differences in the proliferative rates of tumour-associated and normal endothelial cells[103]. New methods for inhibiting VEGFA activity are also being explored; for example, VEGF Trap[104].

Overexpression of the ephrin type A receptor 2 (EPHA2) in tumour cells or endothelial cells has been observed in >75% of ovarian cancers and is associated with increased microvascular density and increased expression of MMP2, MMP9 and MMP14 (Ref. 105). Xenograft growth can be inhibited by agonistic antibodies against EPHA2 (Ref. 106) or by the delivery of EPHA2 siRNA in neutral liposomes[107].

Pericytes contribute to the maintenance of endothelial cells[108] and secrete VEGFA to promote endothelial survival. Their survival, in turn, depends on platelet-derived growth factor (PDGF) produced by endothelial cells. Both endothelial cells and pericytes could thus be targeted by the simultaneous inhibition of VEGFA and PDGF[101]. Trials are planned at the M. D. Anderson Cancer Center to exploit this mechanism for the treatment of ovarian cancer.

Other stromal components

In addition to tumour vessels, ovarian cancers recruit a stroma that contains fibroblasts, lymphocytes and inflammatory cells. Haematopoietic mesenchymal stem cells have been shown to home to ovarian cancer xenografts and differentiate into fibroblasts, contributing to microvascularization, stromal networks and production of tumour-stimulating cytokines (for example, IL-6)[134]. Once recruited, fibroblasts can also be affected by the tumour microenvironment. Ovarian surface epithelial cells that have been immortalized by Ras produce GRO1, which induces the senescence of stromal fibroblasts that produce factors that increase ovarian cancer growth[109].

Lymphocytes and macrophages infiltrate ovarian tumours and are found in ascites in addition to a wide range of cytokines and chemokines[110]. T cells are most prevalent in solid tumour nodules, and their presence is associated with a 5-year survival rate of 38% compared with 4.5% for patients who have tumours that lack T cell infiltrates[111, 112]. A high ratio of CD8$^+$ to regulatory T cells is also associated with an improved prognosis[112]. Interferons produced by activated T cells can inhibit tumour growth and IL-8 secretion, block angiogenesis and upregulate major histocompatibility complex expression, which increases immune recognition. Macrophage colony-stimulating factor and macrophage chemotactic protein 1 secreted by ovarian cancer cells and by T cells are potent chemoattractants for macrophages. Activated macrophages can produce proteins that stimulate (IL-1, IL-6 and TNFα) or inhibit (nitric oxide and TNFα) tumour growth. However, tumour-associated macrophages have impaired phagocytic activity and decreased effector function for antibody-dependent cell-mediated cytotoxicity. The C–X–C chemokine receptor CXCR4 is expressed in 59% of ovarian cancers and is associated with decreased disease-free and overall survival duration; its ligand CXCL12

is found in 91% of ovarian cancers[113, 114,115]. Inhibition of CXCR4 inhibits intraperitoneal dissemination of ovarian cancer xenografts116. Clinical trials should become feasible with the development of potent orally available CXCR4 inhibitors.

Approximately 80% of ovarian cancers express TNFα (which is regulated both translationally and transcriptionally through NF-κB), in addition to TNFRI and TNFRII receptors that allow both autocrine and paracrine stimulation[117]. TNFα can stimulate clonal growth, and TNFα knockdown inhibits growth and dissemination in ovarian cancer xenografts118. Clinical trials have therefore been undertaken with a TNFα-specific antibody for ovarian cancer119.

Conclusion

Given the large number of potential targets in ovarian cancer, an immediate challenge is to identify promising candidates for which inhibition would provide synthetic lethality using conventional drugs or other targeted therapies (Box 4). A crucial goal is to identify patients who would benefit from particular targeted therapies. Given the complexity of crosstalk between protein signalling pathways, predicting the impact and efficacy of any one signalling inhibitor is difficult. Individual inhibitors produce only modest xenograft growth inhibition. Thus, inhibition of multiple pathways will almost certainly be required to substantially affect ovarian cancer growth.

With many new targets and more than 400 potential drugs in the pharmaceutical pipeline, the clinical evaluation of new agents individually, and particularly in combination, is likely to limit the rate of progress. Participation of more ovarian cancer patients, enrichment of Phase I–II trials with cancers that express relevant targets, more efficient Bayesian trial design, use of surrogate biomarkers such as CA125 for response[120], and studying tissues and imaging to determine whether drugs are 'hitting the target' will contribute to accelerating progress in early clinical trials.

As new agents become available, future trials should focus on inhibitors of the molecules (Src, MIS, RAB25 and PKCι) and signalling pathways (PI3K, IL-6–JAK2–STAT3, LPA and NF-κB) that are most frequently activated or overexpressed in ovarian cancers. Targeted therapy should be accelerated by the delivery of siRNAs, which have been shown to be effective in xenograft models of ovarian cancer and are moving towards clinical trials. Greater emphasis should be placed on targeting the interactions of cancer cells with stromal components. In addition to VEGFA, anti-angiogenic therapy could be directed towards other relevant factors (IL-8, PDGF, FGF1, FGF2 and LPA), receptors (EPHA2, PDGFR and FGFR) and cells (pericytes) in combination with metronomic dosing of cytotoxic drugs. If animal models prove to be relevant to the clinic, dormant ovarian cancer cells might be eliminated by targeting autophagy (chloroquine and siRNA) or survival factors (VEGFA, IGF and IL-8) that are required to prevent autophagic death in cancer cells that still express ARHI.

Ultimately, we must improve our ability to predict response in the clinic and to identify patients who are most likely to benefit from particular combinations of drugs. Given the heterogeneity of ovarian cancer at a molecular level, if we are to match the abnormalities present in an ovarian cancer to appropriate therapy, extensive characterization of every cancer specimen must be carried out using DNA sequencing of all relevant genes, high-resolution comparative genomic hybridization, single nucleotide polymorphism analysis, expression arrays, reverse-phase protein arrays and immunohistochemistry of selected biomarkers. As a greater rate of response to targeted therapy might be seen when signalling pathways are activated by genetic events, integrating mutation data from the Cancer Genome Atlas Project and DNA copy number with other 'omics' data should be particularly relevant for defining subsets of patients for therapy. Biopsy samples of fresh ovarian cancer tissue will be required from all patients before Phase

I and II trials. Logistically, this is a particular challenge in recurrent ovarian cancer in which much of the disease is often localized in the abdominal cavity. The reward of more effective, potentially less toxic, individualized targeted therapy provides a substantial incentive to design and execute such trials.

Currently available biomarkers (p53 and KRAS) support the treatment of low-grade serous and non-serous type I and high-grade serous and endometrioid type II ovarian cancers with different agents. Reverse-phase protein array analysis suggests that distinct subsets of patients might benefit from either a combination of PI3K and MAPK inhibitors, a combination of KIT and cyclin E2 inhibitors, hormonal therapy or antiangiogenic approaches. A multi-arm trial is under development to determine the value of predictive tests for assigning patients to each of these treatment groups. As clinical trials mature with PI3K pathway inhibitors, the response in ovarian cancers with gene amplifications (*PIK3CA* and *AKT2*) or mutation (*PIK3CA* and *PTEN*) of pathway components can be compared with cancers in which PI3K activation is driven by autocrine or paracrine stimulation. If mutations are important drivers for cancer cell proliferation and survival, we anticipate that type I ovarian cancers will show a higher response rate than type II cancers to inhibitors of the PI3K–Akt and Ras–Mapk pathways.

In the long term, earlier detection of ovarian cancer is likely to have an important effect on the management of this disease. In the next 3 years, the results of a trial involving 200,000 subjects in the United Kingdom will establish whether measuring increasing CA125 levels followed by transvaginal ultrasound will detect sufficient numbers of patients with early-stage disease to affect survival[121]. Regardless of the outcome, it is likely that multiple marker panels will be required to detect >90% of patients with preclinical or early-stage disease. Understanding the heterogeneity of different ovarian cancers at a molecular level has facilitated the development of candidate panels of markers.

Acknowledgments

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TABLE 1

Genetic abnormalities in epithelial ovarian cancer

Event	Effect	Chromosome	Gene
Gene amplification[‡]	Activation	1q22 3q26 5q31 8q24 19q 20p 20q13.2	*RAB25* *PRKCI, EVI1* and *PIK3CA* *FGF1* *MYC* *PIK3R1* and *AKT2* ND *AURKA*
Gene deletion[‡]	Inactivation	4q, 5q, 16q, 17p, 17q, Xp and Xq	ND
Mutation[§]	Activation	NA	*KRAS* (15%), *BRAF* (12%), *CTNNB1* (12%), *CDKN2A* (10%), *APC* (9%), *PIK3CA* (8%), *KIT* (7%) and *SMAD4* (7%)
Hypomethylation	Activation	NA	*IGF2* and *SAT2*
Loss of heterozygosity	Inactivation	17p13 and 17q21 (in 50% of cases or more) 1p, 3p, 5q, 5q, 6q, 7q and 8q (in fewer than 30% of cases)	*ARHI, PEG3, PLAGL1, RPS6KA2, TP53, BRCA1, BRCA2, PTEN, OPCML* and *WWOX*
Mutation	Inactivation	NA	*TP53* (62%), *BRCA1* (5%), *BRCA2* (<5%) and *PTEN* (3–8%)
Promoter methylation[#]	Inactivation	NA	*ARHI, DAPK1, CDH13, MLH1, ICAM1, PLAGL1, DNAJC15, MUC2, OPCML, PCSK6, PEF3, CDKN2A, CDKN1A, RASSF1, SOCS1, SOCS2, PYCARD* and *SFN*

The growth of ovarian cancer can also be induced by transfer of chromosomes 2, 3, 7 and 22.

[*] Table adapted from REF. [11]

[‡] Determined by array comparative genomic hybridization.

[§] Data obtained from http://www.sanger.ac.uk.

[#] Expanded from REF. [29]. *APC*. adenomatous polyposis coli; *AURKA*, aurora kinase A; *CDH13*, cadherin 13; *CDKN*, cyclin-dependent kinase inhibitor; *CTNNB1*; β-catenin 1; *DAPK1*, death-associated protein kinase 1; *EVI1*, ecotropic viral integration site 1; *FGF1*, fibroblast growth factor 1; *ICAM1*, intracellular adhesion molecule 1; *IGF2*, insulin-like growth factor 2; *MUC2*, mucin 2; NA, not applicable; ND, not determined; *OPCML*, opioid-binding protein/cell adhesion molecule-like; *PCSK6*, proprotein convertase subtilisin/kexin type 6; *PEG3*, paternally expressed 3; *PIK3CA, PI3K* catalytic subunit-α; *PIK3R1*, PI3K regulatory subunit 1; *PRKCI*, protein kinase Cι; *PLAGL1*, pleiomorphic adenoma gene-like 1; *RPS6KA2*, ribosomal protein S6 kinase 2; *SAT2*, spermidine/spermine N1-acetyltransferase family member 2; *SFN*; stratifin; SOCS, suppressor of cytokine signalling.

TABLE 2

Putative tumour suppressor genes of which function is lost in epithelial ovarian cancer

Gene	Chromosome	Percentage of cancers in which downregulated or inactivated	Mechanisms of downregulation	Function
ARHI (DIRAS3)	1p31	60%	Imprinting; LOH; promoter methylation; transcription downregulated by E2F1 and E2F4	26 kDa GTPase; inhibits proliferation and motility; induces autophagy and dormancy; upregulates P21; inhibits cyclin D1, PI3K, Ras–Mapk signalling and STAT3
RASSF1A	3p21	60%	Hypermethylation	Inhibits proliferation and tumorigenicity in many different cancers; interacts with Ras inhibiting and downregulating cyclin D and signalling through JNK; stabilizes microtubules; regulates spindle checkpoint; regulates CD95- and TNFα-induced apoptosis
DLEC1	3p22.3	73%	Promoter hypermethylation and histone hypoacetylation	166 kDa cytoplasmic protein that inhibits anchorage-dependent growth
SPARC	5q31	70–90% decreased expression; 9% lost expression	Transcription	32 kDa Ca^{2*}-binding protein; prevents adhesion
DAB2 (DOC2)	5q13	58–85% lost expression	Transcription	105 kDa protein binds GRB2, preventing Ras and Mapk activation; prevents FOS induction and decreases ILK activity; contributes to anoikis; inhibits proliferation; inhibits anchorage-independent growth and tumorigenicity
PLACL1 (LOT1)	6q25	39%	Imprinting; LOH; transcription downregulated by EGF end TPA	55 kDa nuclear zinc-finger protein; inhibits proliferation and tumorigenicity
RPS6KAZ	6q27	64%	Monoallelic expression in ovary; LOH	90 kDa ribosomal S6 serine threonine kinase; inhibits growth; induces apoptosis; decreases Erk phosphorylation and cyclin D1; increases p21 and p27
PTEN	10q23	3–8% mutated; expression lost in 27%, particularly in endometrioid and clear-cell histotypes	Promoter methylation; LOH; mutation	PI3 phosphatase; decreases proliferation, migration and survival; decreases cyclin D; increases p27
OPCML	11q25	56–83%	Promoter methylation; LOH; mutation	GPI-anchored IgLON family member induces aggregation; inhibits proliferation and tumorigenicity
BRCA2	13q12–13	3–6%	Mutation; LOH	Binds RAD51 during repair of DNA DSBs
ARL11	13q14	62%	Promoter methylation	ADP ribosylation factor; induces apoptosis
WWOX	16q23	30–49%, particularly in mucinous end clear–cell histotypes	LOH; mutation	Decreases anchorage-independent growth and tumorigenicity; mouse homologue required for apoptosis
TP53	17p13.1	50–70%	Mutation	53 kDa nuclear protein; induces p21 leading to cell cycle arrest

Gene	Chromosome	Percentage of cancers in which downregulated or inactivated	Mechanisms of downregulation	Function
				and promotion of DNA stability; induces apoptosis
DPH1	17p13.3	37%	LOH	50 kDa protein; decreases proliferation and clonogenicity; decreases cyclin Dl
BRCA1	17q21	6–8%	Mutation; LOH	E3 ubiquitin ligase that participates directly in repair of DNA DSBs through homologous recombination; regulates ABL1; induces p53, androgen receptor, oestrogen receptor and MYC
PEG3	19q13	75%	Imprinting; LOH; promoter methylation; transcription	Induces p53-dependent apoptosis

*Table adapted from REF. [11] *ARL11*. ADP-ribosylation factor-like 11; *DAB2*. disabled homologue 2; *DLEC1*. deleted in lung and esophageal cancer 1; DSB. double strand break; EGR, epidermal growth factor; CPI, glycosylphosphatidylinositol; GRB2. growth factor receptor-bound 2; ILK, integrin-linked kinase; JNK. JUN N-terminal kinase; LOH, loss of heterozygosity; *OPCML*, opioid-binding protein/cell adhesion molecule-Like; *PEG3*, paternally expressed 3; *PLAGL1*, pleiomorphic adenoma gene-like 1; *RPS6KA2* ribosomal protein S6 kinase 2; *SPARC*. secreted protein, acidic, cysteine-rich; STAT3, signal transducer and activator of transcription 3; TNFα, tumour necrosis factor-α; TPA, tissue plasminogen activator.

TABLE 3

Oncogenes associated with epithelial ovarian cancer

Gene	Chromosome	Percentage of cancers in which amplified	Percentage of cancers in which overexpressed	Percentage of cancers in which mutated	Function
RAB25	1q22	54%	80–89%	ND	Cytoplasmic GTPase and apical vessel trafficking
EVI1	3q26	ND	ND	ND	Transcription factor
EIF5A2	3q26	ND	ND	ND	Elongation factor
PRKCI	3q26	44%	78%	ND	Cytoplasmic serine–threonine protein kinase
PIK3CA	3q26	9–11%	32%	8–12%	Cytoplasmic lipid kinase
FGF1	5q31	ND	51%	ND	Growth factor for cancer and angiogenesis
MYC	8q24	20%	41–66%	ND	Transcription factor
ECFR	7q12	11–20%	9–28%	<1%	Protein tyrosine kinase growth factor receptor
NOTCH3	9p13	20–21%	62%	ND	Cell surface growth factor receptor
KRAS	12P11–12	5%	30–52%	2–24%	Cytoplasmic GTPase
ERBB2	17q12–21	6–11%	4–12%	ND	Protein tyrosine kinase growth factor receptor
PIK3R1	19q	ND	ND	ND	Cytoplasmic lipid kinase
CCNE1	19q12	12–36%	42–63%	ND	Cyclin
AKT2	19q13.2	12–27%	12%	ND	Cytoplasmic serine–threonine protein kinase
AURKA	20q13	10–15%	48%	ND	Nuclear serine–threonine protein kinase

*
Table adapted from REF. 11. *AURKA*, aurora kinase A; *CCNE1*, cyclin E1; *EGFR*, epidermal growth factor receptor; *EIF5A2*, eukaryotic translation initiation factor 5A2; *EVI1*, ecotropic viral integration site 1; *FGF1*, fibroblast growth factor 1; *PIK3CA*, PI3K catalytic subunit-α; *PIK3R1*, PI3K regulatory subunit polypeptide 1; *PRKCI*, protein kinase Cι.