Chapter 2
Discovery of Novel Targets

John Farley and Michael J. Birrer

Abstract Ovarian cancer has the highest case fatality rate of any gynecologic cancer. Thus, intense efforts are being dedicated to identifying new therapeutic targets and pathways which will provide new therapeutic approaches. Historically, this approach has involved the empiric testing of agents in clinical trials attempting to identify one with global activity against cancers. This process has been difficult, expensive, and time-consuming. For ovarian cancer, it has produced a homogenous approach to all forms of ovarian cancer. More recently, however, with the revolution in molecular technologies there has been a major change in our ability to rationally identify therapeutic targets in these tumors. This chapter will review the application of these new technologies to ovarian cancer. Genomic discoveries have revealed a remarkable heterogeneity within ovarian cancers and the diverse molecular pathways found in these tumors provide a molecular underpinning of their clinicopathologic characteristics. Using a systematic assessment of these molecular details with an algorithm of filtering and biomarkers validation, it will be possible to identify and eventually exploit new and novel therapeutic targets within these cancers. This will ultimately deliver more individualized care.

Keywords Gynecologic · Malignancies · Therapy

2.1 Introduction

Ovarian cancer remains an important health problem for women in the United States. Ovarian cancer has the highest case fatality rate of any gynecologic cancer and it is the most common cause of death from cancers of the female genital tract

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[1–3]. The high case fatality rate results from the frequent diagnosis of epithelial ovarian cancer at an advanced stage: 75% of all cases are diagnosed as stage III or IV, where the disease has spread throughout the abdomen. Improvements in surgical approach with extensive debulking and the use of platinum-based regimens have dramatically extended progression-free survival. Unfortunately, up to 75% of patients with advanced-stage disease will develop recurrent disease, which rapidly acquires chemoresistance leading to death from disease [1–4]. Patients with advanced-stage disease have a 5-year survival of only 29% with little improvement in overall survival over the last 30 years.

Thus, intense efforts are being dedicated to identifying new therapeutic targets and pathways which will provide new therapeutic approaches. Historically, this approach has involved empiric testing of new agents in phase II and III trials attempting to identify agents with global activity against the majority of ovarian cancers. This process has been difficult, expensive, and time-consuming. More recently with the cloning of the human genome and the development of high-throughput technologies, there has been a revolution in our ability to identify rational targets in these tumors. Through a better understanding of the molecular origins of tumors and underlying basis for their clinicopathologic characteristics, novel therapeutic targets can be established through a more rational process. The genomic characterization of tumors allows for the delineation of differentially expressed genes, amplified genes, methylated genes, and ultimately activated pathways. This new approach will revolutionize our ability to identify new effective agents for the treatment of ovarian cancer.

2.2 The Historic Perspective of Drug Development – Empirical Approaches

The origin of cytotoxic chemotherapy dates back to 1946 when Goodman described the effectiveness of nitrogen mustard in the treatment of human malignancies in the *Journal of the American Medical Association* [5]. One of the first compounds used in the treatment of gynecologic malignancy was methotrexate. This application of methotrexate in the treatment of gynecologic malignancies began in the 1950s when Dr. Min C. Li evaluated methotrexate in the treatment of a patient with melanoma that was unsuccessful; however, Dr. Li noted that elevated levels of urine hCG fell dramatically with methotrexate treatment [6]. Dr. Li and Dr. Roy Hertz subsequently administered methotrexate to a woman with metastatic choriocarcinoma, initially with a palliative intent [6]. A decrease in β-HCG levels was noted, followed by complete clinical resolution of tumor burden. This heralded the treatment of choriocarcinoma with methotrexate.

While the above chemotherapeutic agents provided advances to the field of oncology as a whole, it was not until the use of platinum-based agents that patients with gynecologic malignancies (most notably ovarian cancer) appreciated an improvement in survival. The antiproliferative properties of platinum co-ordination

complexes were observed in 1965, which was followed by the first report of cisplatin's antitumor effect in 1974 [7]. This substantial single-agent effect of cisplatin was documented in both testicular and ovarian cancer, with objective responses reported in 3/7 and 7/19 patients, respectively. Standard of care chemotherapy for ovarian epithelial cancer also includes the addition of a taxane chemotherapeutic agent [4]. Isolated from the bark of the Pacific yew, *Taxus brevifolia*, Taxol was initially identified as a cytotoxic agent in a screen of natural products sponsored by the National Cancer Institute. The addition of a taxane to platinum chemotherapy has improved survival for advanced ovarian cancer patients [4]. Further, gemcitabine (2′2′-difluorodeoxycytidine, dFdC) is a nucleoside analogue of cytidine which has also demonstrated activity in ovarian cancer [8].

The historic approach to the development of chemotherapeutic agents has been essentially empiric in nature and has not been tailored to specific biologic or pathologic aspects of the tumor. This has resulted in a "one size fits all" for ovarian cancer in that all ovarian cancers are treated with essentially the same therapeutic agents. However, it has been noted for a long time that ovarian tumors span a spectrum of histologies and tumor grade. The biology and clinical behavior of these specific tumors remains quite different. The recent application of molecular technology to ovarian cancers has provided major advances in understanding the biology of these malignancies and has revealed a dramatic molecular heterogeneity within ovarian cancers. This technology has revealed the underlying molecular basis for differences of histology and tumor grade. Further, it has the potential to identify many new and novel targets including cell cycle regulators, growth factor receptors, signal transduction proteins, and molecules that confer drug resistance, induce tumor progression, and promote angiogenesis

2.3 Genomics

The development of advanced genomic technologies, such as oligonucleotide microarray analysis, has provided a means to capture global gene expression patterns for a large number of tissue samples. Oligonucleotide microarrays have the capability to determine the expression of all the genes expressed within a cell simultaneously [5, 8]. This gene expression pattern can be correlated with many clinically relevant characteristics of an individual tumor. One of the most common array platforms is the Affymetrix® expression platform in which total RNA is extracted and purified. Biotin-labeled cRNA is then prepared for each sample. Labeled cRNA is fragmented, combined with a hybridization cocktail containing biotinylated hybridization controls, and incubated on the oligonucleotide array. Laser excitation then stimulates fluorescence emission of labeled probes bound to target sequences, creating a specific image for the sample analyzed. Array images are then acquired and analyzed with GeneChips Operating Software (GCOS).

Oncologists are beginning to investigate a variety of new biologic agents for the treatment of ovarian cancer [9, 10]. The ideal molecular target for clinical therapeutic applications should be differentially expressed by the tumor, have a potential

druggable molecular site, and be necessary for the viability of the cancer cell [11]. The molecular heterogeneity of ovarian cancer compared to other disease sites, such as hematologic malignancies, has made the successful transfer of molecular agents into the ovarian cancer treatment armamentarium problematic [11]. As opposed to the singular molecular abnormality observed in GIST (*c-kit*) or CLL (*BCR-ABL*) for which imatinib (Gleevec) is effective, ovarian cancer possesses a multitude of molecular abnormalities any of which may play a pivotal role in ovarian cancer proliferation and survival. An appreciation and understanding of the complex pathways of growth deregulation in gynecologic cancers is providing a framework for the rational application and testing of novel therapies [9, 10]. Molecular classification of ovarian cancer could allow for the same stratification and treatment.

2.4 Tumor Histology

There is substantial evidence that cellular morphology (histology) might affect clinical responses to ovarian cancer. Although the clinical approach to epithelial ovarian cancer is quite uniform with all patients being treated with standard surgery and chemotherapy, there is in fact considerable clinicopathologic heterogeneity among the tumors. The most common histology of ovarian cancer is papillary serous (50–60% of all cancers), while less common histologies include endometrioid (25%), clear cell (4%), and mucinous (4%) [12]. Papillary serous and endometrioid tumors frequently present at advanced-stage disease, having spread throughout the abdomen [12]. In contrast, clear cell and mucinous tumors tend to present as tumors limited to one or both of the ovaries and can be amendable to complete surgical resection [3, 13]. Even in advanced-stage disease, there are notable differences among histology types, with papillary serous and endometrioid tumors being very chemoresponsive (75% response rate) while mucinous and clear cell are considerably more resistant to standard therapy (35% response rate) [13–16]. The use of genomics technology has provided significant improvement in the classification of tumors by comprehensive molecular analysis.

In an effort to further identify the molecular signatures of the specific ovarian cancer histologies, the gene expression profiles of serous, endometrioid, and clear cell cancers were examined [17]. A total of 24 papillary serous, 11 endometrioid, and 9 clear cell ovarian tumors were analyzed. Comparing the histosubtypes of ovarian cancer directly to one another, 166 genes differentiated the samples into the 3 subtypes. When clear cell ovarian cancer was compared with non-clear cell ovarian cancer (serous and endometrioid ovarian cancers grouped together), 171 differentially expressed genes were identified. Serous and endometrioid cancers were distinguished from the other histologic subtypes by 62 and 66 differentially expressed genes, respectively [17].

To identify specific genes involved in the development of the individual histologic types of ovarian cancers, separate comparisons of each histologic subtype to normal OSE brushings were completed. These comparisons yielded lists of 94 genes for clear cell cancer, 422 genes for endometrioid cancer, and 467 genes for serous

cancer [17]. Forty-three genes were common to all three lists and therefore displayed consistent differential expression between normal OSE and ovarian cancer regardless of histologic subtype. Twenty-nine genes have increased expression in ovarian cancer compared with normal OSE, whereas 14 have decreased expression in cancer. Among the genes with increased expression in cancer are homogentisate oxidase (HGD), peroxisome proliferative-activated receptor gamma (PPARG), v-rel reticuloendotheliosis viral oncogene homologue B (RELB), and p21-activated kinase 1 (PAK1) [6]. Decreased expression was documented for tenascin XB (TNXB), galectin 8 (LGALS8), post-meiotic segregation increased 2-like 8 and 2-like 9 (PMS2L8 and PMS2L9), deafness autosomal dominant 5/inversely correlated with estrogen receptor expression 1 (DFNA5/ICERE1), disabled homologue 2/differentially expressed in ovarian cancer 2 (DAB2/DOC2), and retinoic acid receptor responder 1 (RARRES1/TIG1) [17].

This group of 43 genes comprised the common genes appearing on each ovarian cancer subtype's comparison with normal OSE. This suggests that at least part of the transformation process might be shared among endometrioid, serous, and clear cell ovarian cancers, as evidenced by the common genes distinguishing them from normal OSE. However, the question of whether the OSE serves as a common precursor is not necessarily clarified. It is conceivable that tumors of different histologies may arise from different precursor cells but undergo similar transformation events.

Of great interest is the fact that when individual histotypes from different organs are compared, both serous and endometrioid cancers can be separated based upon the organ of origin. However, clear cell cancers were indistinguishable based upon their gene expression patterns (Fig. 2.1). In fact, clear cell cancer from the ovary and endometrium could not be distinguished from clear cell cancers from the kidney [17]. These data strongly support the hypothesis that clear cell cancers are unique in that they arise through a similar developmental process or cell of origin regardless of organ site. This has significant clinical implications in that clear cell cancers should be considered a separate disease and that the identification of effective therapies should be achieved by phase II trials specific for these tumors.

2.5 Genomic Analysis Reveals Heterogeneity Within Ovarian Tumors Based upon Tumor Grade

The histologic grade of epithelial ovarian tumors spans the spectrum from low malignant potential tumors (grade 0) to low-grade invasive cancers (grade 1) to high-grade tumors (grades 2 and 3). The relationship between these tumors has remained somewhat unclear. Borderline tumors (or LMP tumors) have the features of malignancy including nuclear atypia and abnormal nuclear/cytoplasmic ratios but lack the highly aggressive, metastatic phenotype of higher grades of epithelial ovarian cancer. The biological relationship among LMP tumors, low-grade, and high-grade invasive serous ovarian carcinomas was analyzed in 90 microdissected serous ovarian tumors which spanned the aforementioned serous pathologic spectrum and also included normal ovarian surface epithelium (OSE) brushings. These tumors were

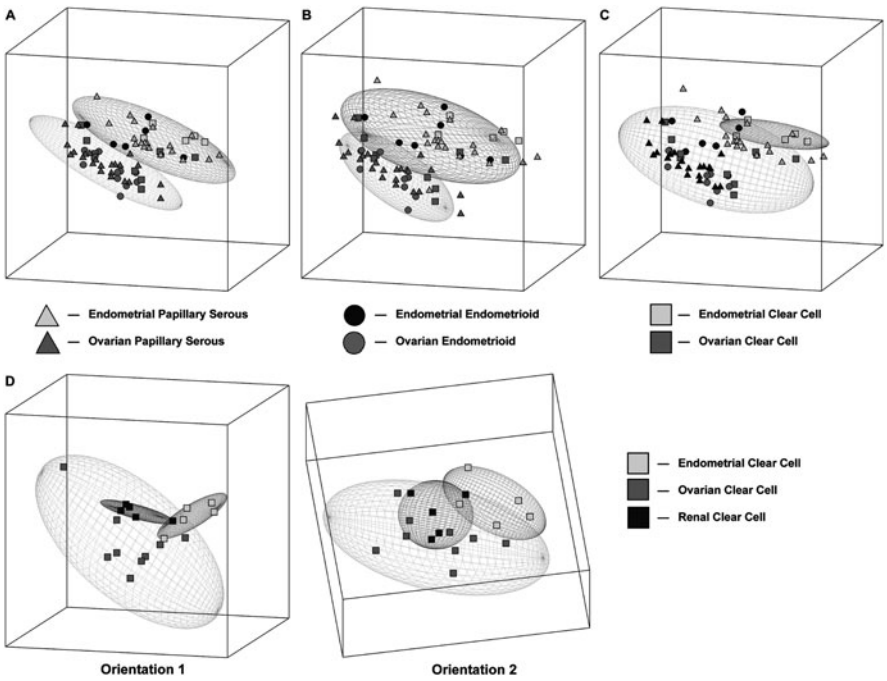

Fig. 2.1 Principal component analysis (PCA) of ovarian and endometrial cancers according to histology. **a** PCA of tumors with serous histology showing two non-overlapping elliptical regions separating endometrial (*top*) from ovarian (*bottom*) specimens. **b** PCA of tumors with endometrioid histology showing two non-overlapping elliptical regions separating endometrial (*top*) from ovarian (*bottom*) specimens. **c** PCA of tumors with clear cell histology showing overlapping elliptical regions representing endometrial (*top*) and ovarian (*bottom*) specimens. **d** PCA of tumors according to organ of origin shows three overlapping elliptical regions among ovarian, endometrial, and renal clear cell specimens, with two different orientations (1 and 2)

interrogated using the 47,000 transcript Affymetrix U133 Plus 2.0 oligonucleotide array [5]. Unsupervised analysis showed a distinct separation between LMP tumors and high-grade cancer (Fig. 2.2a). Furthermore, when low-grade invasive tumors were included in the analysis, they closely aligned with LMP lesions rather than their high-grade invasive counterparts (Fig. 2.2b). The identification of two unique branches containing LMP tumors and high-grade carcinomas is consistent with the distinct clinicopathologic aspects of the two diseases and prior molecular studies [5, 13, 14]

2.6 Bioinformatic Analysis Reveals Activated Pathways Within LMP and Low-Grade Ovarian Cancer

Using bioinformatic approaches to genomic data has provided critical information to identify activated pathways in these tumors. Signaling pathways contributing to the phenotype associated with LMP tumor have been identified in 773 and 1,755

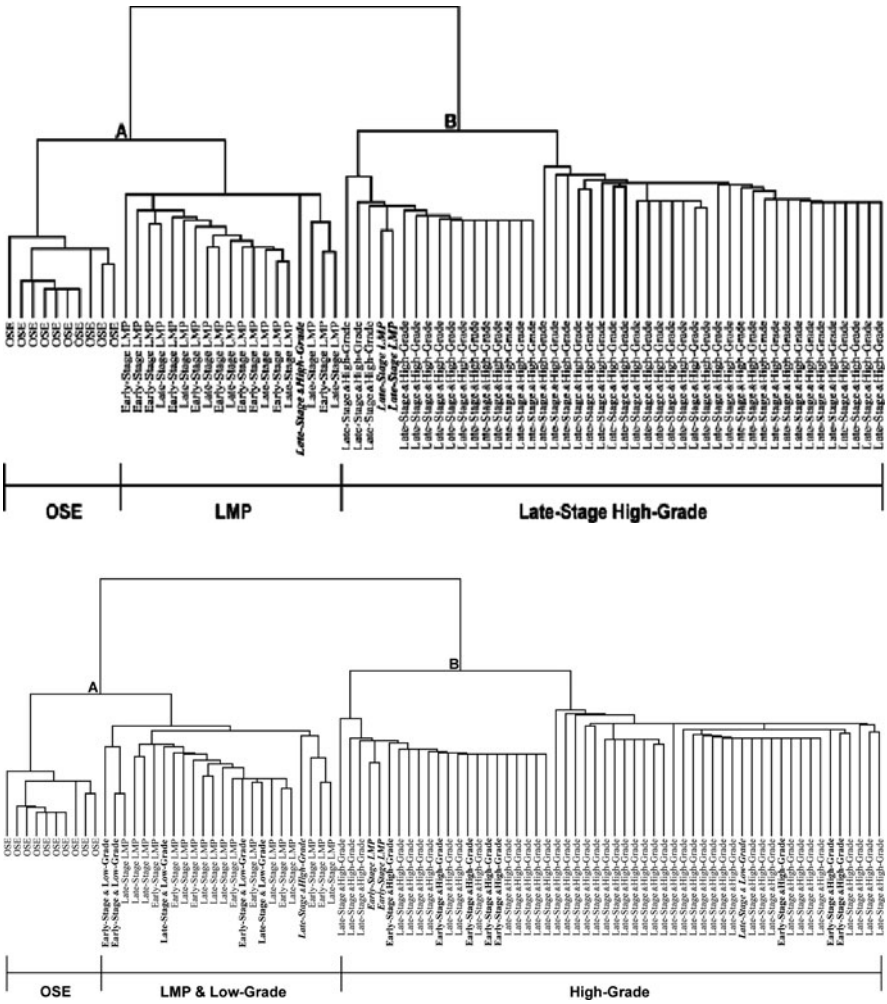

Fig. 2.2 **a** Hierarchical clustering analysis of the 16,178 probe sets passing the filtering criteria for LMP tumors, late-stage, high-grade cancers, and OSE. OSE specimens grouped independently from LMP specimens (*node A*), whereas late-stage, high-grade tumors clustered in two distinct groups (*node B*). Misclassified specimens are *bold italicized*. **b** Hierarchical clustering analysis of the 14,119 probe sets passing the filtering criteria for LMP, low-grade, high-grade, and OSE specimens and binary tree validation. Overall tree structure was retained despite the association of low-grade tumors with LMP tumors and the grouping of early-stage and late-stage, high-grade lesions. Low-grade and early-stage, high-grade samples are indicated in *bold*. Misclassified specimens are *bold italicized*

unique genes differentially regulated in LMP and late-stage, high-grade tumors versus OSE, respectively [18]. Thirteen differentially regulated genes specific to LMP tumors encoded proteins that were associated with TP53-mediated repression of cell proliferation and promotion of senescence as well as the stabilization of CDKN1A. LMP tumors as would be expected clinically did not show any of the pathways

involving cellular proliferation, metastasis, and chromosomal instability identified within high-grade invasive tumors [18].

In contrast, growth control pathways, such as the p53 pathway, characterized LMP tumors. For instance, two negative regulators of p53, UBE2D1 and ADNP, are downregulated in LMP tumors. UBE2D1 is an ubiquitin-conjugating enzyme that can target p53 for degradation by the proteasome, whereas antisense oligonucleotide knockdown of ADNP in intestinal cancer cells can upregulate p53 expression and diminish cancer cell viability [18, 19]. In addition, elevated expression of PPM1A, found in LMP tumors, leads to G2–M cell cycle arrest through increased expression of p53 and its downstream target p21 [18, 20].

In LMP tumors, decreased expression of these genes may bolster the antiproliferative activity of p21. The concerted deregulation of these genes leads to activation of the p53 pathway and upregulation of p53-regulated downstream genes. Activated p53 can inhibit CDC2, PCNA, STMN1, and EZH2, all of which are overexpressed in high-grade lesions and are associated with transformation [18, 21–23]. Furthermore, p53-mediated expression of PML and GDF15 may play an essential role in promoting terminal differentiation and restricting cellular proliferation [24]. Taken together, these differentially expressed genes may account in part for the more limited proliferative capacity attributed to LMP lesions. The assignment of low-grade invasive tumors within the LMP branch argues that these invasive tumors are more similar to LMP tumors than high-grade lesions. Indeed, absent in LMP tumors *and* low-grade invasive tumors are pathways implicated in cell cycle progression, cellular proliferation, and chromosomal instability seen in high-grade tumors [18]. In addition, there are other differentially regulated genes common to LMP tumors and low-grade cancers, which may also contribute to the proliferative phenotype associated with these tumors.

It is important to note that there are significant differences between LMP tumors and low-grade invasive cancers. The expression profiles for invasive low-grade tumors do not contain the enhanced p53-signaling activity observed in LMP tumors [18]. Whereas RHOA and ITGB1 were co-regulated in low-grade tumors, other members involved in p53 signaling were not differentially expressed. Interestingly, PDCD4 and CCNPB1 were downregulated in low-grade tumors. Both of these genes are implicated in cell cycle progression and were co-regulated in high-grade lesions. Differential regulation of these genes may contribute to the development of this invasive tumor and somewhat clinically aggressive nature of low-grade tumors when compared to LMP tumors. RT-PCR confirmation of p53 regulators ADNP and UBE2D1, as well as p53 effector GDF15, in LMP but not low-grade tumors substantiates this observation. These alterations may partially mediate the transition from a low proliferative LMP or non-invasive MPSC to an invasive low-grade lesion.

As discussed by Shih and Kurman, it is conceivable that invasive low-grade tumors may arise from non-invasive, low proliferative LMP lesions [25]. Low-grade carcinomas are clinically typified by nuclear atypia, which are distinct from high-grade lesions [26]. They also follow an indolent course that may extend >20 years [25]. Several lines of molecular evidence support this model, including an increased frequency of KRAS and BRAF mutations, an absence of TP53 mutations,

low cellular proliferation, and a gradual increase in chromosomal instability among LMP, MPSC, and low-grade lesions [18, 27]. There are also clinical data showing the existence of recurrent low-grade carcinoma in patients initially diagnosed with LMP disease [28]. If LMP tumors possess the ability to develop into low-grade lesions, the progression from LMP to low-grade cancer may involve the attenuation of p53 signaling.

In summary, the expression profiles generated for LMP, low-grade, and high-grade papillary serous ovarian carcinomas show a close association between LMP and low-grade lesions. Prominent expression of TP53, CDKN1A, and other p53-modulated genes in LMP tumors suggests that this signaling pathway may play an important role in the distinct phenotype associated with this lesion [18]. Furthermore, a return of TP53 and CDKN1A to levels expressed in OSE may precede progression of these low proliferative cancers to more aggressive low-grade tumors. Targeting deregulated genes that are repressed in high-grade cancers for therapeutic intervention may attenuate the progression of the disease.

2.7 High-Grade Ovarian Cancer

High-grade serous ovarian cancers appear pathologically homogeneous. However, it is important to note that there are subsets of patients displaying distinct clinical phenotypes (e.g., survival or chemoresponse). It is likely that this spectrum of clinical outcomes is driven by unique genes/pathways. Genomic approaches can identify genes whose expression correlates with clinical characteristics of these tumors. This approach can identify novel therapeutic targets. For instance, in a recent study whole-genome oligonucleotide array was used to perform expression profiling on a series of microdissected late-stage, high-grade papillary serous ovarian adenocarcinomas in order to identify a prognostic gene signature (prediction analysis) correlating with survival as a continuous variable. Fifty-three advanced-stage, high-grade primary tumor specimens from patients with papillary serous adenocarcinomas of the ovary, whose survival spanned a spectrum of 145 months, were included in this analysis. All specimens were subjected to laser-based microdissection and analyzed as pure, microdissected epithelial cell populations on whole-genome Affymetrix U133 Plus 2.0 GeneChip microarrays. The performance of the prediction analysis was visualized by hierarchical clustering, which demonstrated the ability of the top scoring genes (Cox hazard ratio > 10) to cluster the 53 specimens according to survival (Fig. 2.3). The validity of the entire 200 probe set classifier was then evaluated by a non-parametric log-rank test using median hazard to stratify the patients. The test was highly significant, with the high-risk group, defined by predicted hazard greater than median hazard, having a significantly shorter survival than the low-risk group (Fig. 2.1c). Independent evaluation confirmed the association of a prognostic gene, microfibril-associated glycoprotein 2 (MAGP2), with poor prognosis (Fig. 2.3b) [29]. MAGP2 is a 25-kDa matrix glycoprotein, which was originally identified by its co-extraction

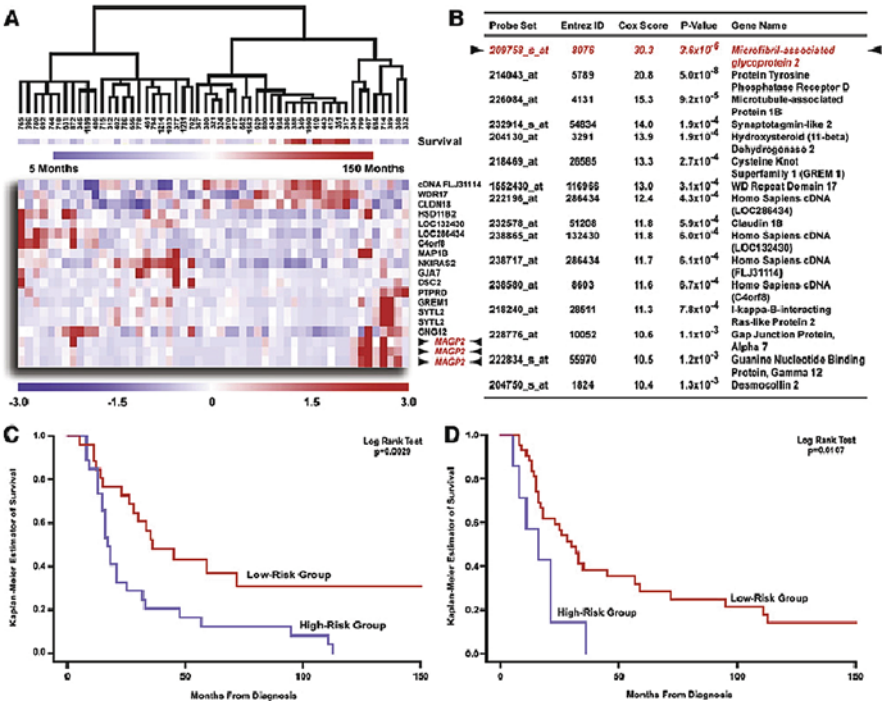

Fig. 2.3 **a** Hierarchical clustering of 53 advanced-stage, high-grade serous adenocarcinomas using expression values for genes possessing a Cox score > 10 (gene expression: *red*, upregulated; *blue*, downregulated; survival: *blue*, short survival; *red*, long survival). **b** Genes presented in this table possessed a large Cox score (>10). Only the probe set with the highest Cox score is presented for MAGP2. **c** Kaplan–Meier analysis of the predictor demonstrated a significant difference in survival time ($p = 0.0029$). **d** Kaplan–Meier survival analysis of 49 patients using qRT-PCR validation data obtained for the top 11 survival-signature genes confirmed that the two groups retained significantly different survival endpoints ($p = 0.0107$)

from developing fetal nuchal ligament tissue [29, 30]. However, it has never been identified or evaluated in the context of ovarian cancer.

Prognostic genes identified in this manner can then be placed in biologic context by a bioinformatics approach. For instance, to ascertain whether subsets of the survival-associated genes might be participating in co-ordinated signaling pathway(s) contributing to patient outcome, 53 advanced-stage ovarian adenocarcinoma specimens were compared to 10 normal ovarian surface epithelium (OSE) brushings. Integrin-mediated signaling stimulated by MAGP2 engagement of the $\alpha_V\beta_3$ receptor featured prominently in the analysis and a number of downstream effectors were overexpressed including PXN, FAK, GRB2, and SOS1. Contributing to this pathway was a number of genes implicated in patient survival including MAGP2, FGF18, FGFR2, ADAM12, NEDD9, MMP13, and CDC2. Of these, MAGP2, FGF18, FGFR2, and CDC2 were also significantly upregulated [29].

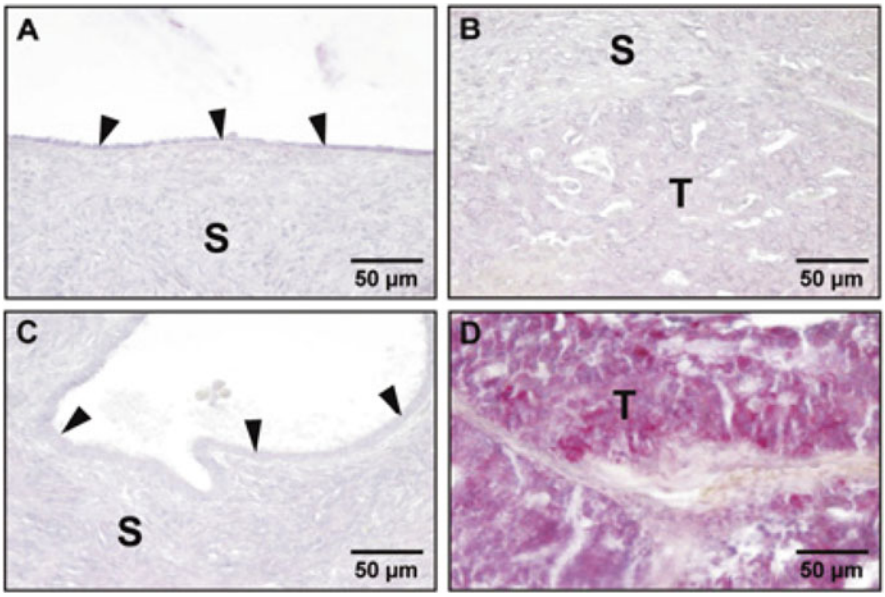

Fig. 2.4 Low-level MAGP2 protein staining was observed in normal surface epithelia (**a**), epithelial and stromal components of benign ovarian cysts (**c**), and in some high-grade serous tumors (**b**). Strong MAGP2 staining was observed in a small proportion of high-grade serous ovarian tumor tissues (**d**). Arrowheads indicate the epithelial layer of the normal ovary and benign ovarian cyst (S, stroma; T, tumor cells)

Immunolocalization of MAGP2 protein in optimally debulked stage III grade 3 serous adenocarcinoma demonstrated low-level expression of MAGP2 in normal ovarian epithelial cells (Fig. 2.4a) and benign cysts (Fig. 2.4c), but elevated levels in a proportion of malignant tumors (Fig. 2.4b, d). The intensity of MAGP2 staining indicated that patients positive for MAGP2 possessed a poor prognosis (Fig. 2.5a). qRT-PCR analysis using all tumor isolates confirmed the association between MAGP2 mRNA expression and patient survival (Fig. 2.3b). Independent validation of this association was completed with a 64-element tissue microarray (TMA) consisting of advanced-stage, high-grade serous adenocarcinoma specimens. MAGP2 staining intensity correlated with survival and adjusted for debulking status by multivariate Cox regression analysis. A significant association was found between the degree of MAGP2 staining and survival (hazard ratio 1.857; $p = 0.004$; 95% confidence interval [1.253 and 2.752]).

Thus, using these data a prognostic gene signature of biological significance in the treatment of epithelial ovarian cancer has been developed. Within the signature are important therapeutic targets. MAGP2 may serve as a survival-associated target. Resistance to chemotherapy has been linked to $\alpha_V\beta_3$ signaling in a number of tumor systems including ovarian cancer [29, 31]. Consequently, stimulation of the receptor by MAGP2 may attenuate chemoresponse, ultimately affecting patient survival. In fact, MAGP2 expression levels were significantly lower in patients who

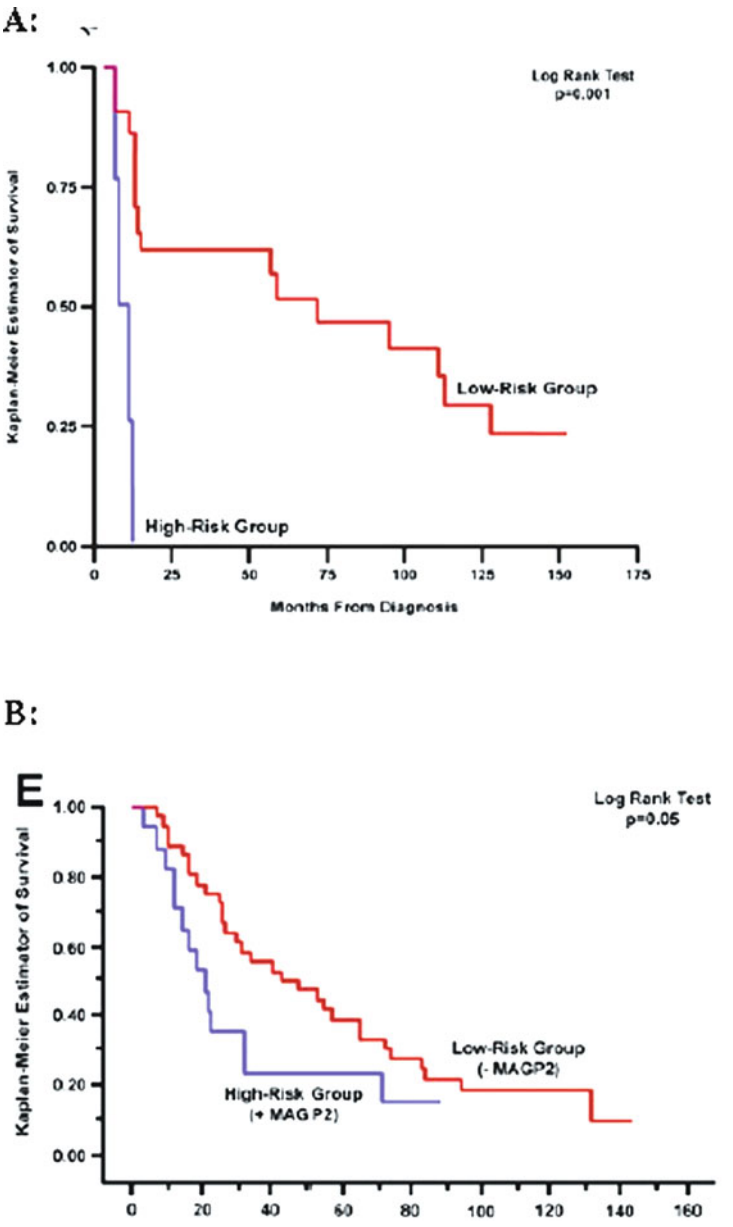

Fig. 2.5 **a** Kaplan–Meier survival analysis of MAGP2 protein expression using 53 patients with stage III/IV high-grade serous ovarian cancer. A statistically significant difference was observed between the outcome groups ($p = 0.05$). **b** Kaplan–Meier survival analysis of MAGP2 mRNA expression using the 53 patients with stage III/IV high-grade serous ovarian cancer demonstrated a significant difference in patient outcome ($p = 0.001$)

responded to chemotherapy. Further mechanistic studies have revealed that MAGP2 can stimulate the migration and invasion of endothelial cells. This raises the possibility that MAGP2 is a proangiogenic factor in ovarian cancer. Indeed, there is a direct correlation of MAGP2 expression with microvessel density within serous cancers. In addition, localization to the extracellular matrix makes it an attractive target for therapeutic intervention.

2.8 Future Directions in the Identification of Novel Therapeutic Targets

A priority translational research objective in gynecologic cancer research should be the discovery of novel therapeutic targets. Ideally, co-discovery of predictive biomarkers occurs in parallel to facilitate clinical development of agents and ultimately personalize clinical use. As demonstrated above, genomic discoveries can be utilized to identify novel therapeutic targets in these tumors. The key element will be the effective usage of these data and the creation of a method to select and prioritize these targets. This process will require target discovery, clinical correlation, validation, mechanistic delineation, prospective testing, and therapeutic exploitation [32] (Fig. 2.6). Significant progress has been made toward formalizing this process. The discovery phase is well developed with multiple genomic analyses performed by many different laboratories including consortium such as The Cancer Genome Atlas (TCGA) Project. These genomic discovery efforts have utilized multiple platforms including expression profiling; copy number differences, methylation patterns, and most importantly direct RNA sequencing. The next step will involve correlation with clinical endpoints such as patient survival, tumor recurrence, or response to chemotherapy. Biomarkers that pass this step will then be validated using fully independent sets of tumors and exploration for a mechanistic and biologic basis. This process will involve an exchange between the validation process and the biologic mechanism exploration so as to identify the highest priority targets. The validation of targets will involve quantitative RT-PCR (qRT-PCR), IHC, methylation-specific PCR, or direct sequencing depending upon the target and a component of this assessment will involve bioinformatic analysis to place the biomarker in the appropriate biologic context. These data will be integrated into targets which make biologic sense in terms of tumor growth, spread, and response to chemotherapy. Targets which pass this analysis will then be validated using prospectively collected clinical trial specimens. These high-annotated specimens from carefully controlled trials will provide the highest level of scrutiny needed to provide the best target choices. The selected list of targets would then be exploited using small molecule inhibitors or antibodies in clinical trials. Figure 2.6 provides a schematic for the novel target development process and emphasizes the complexity of the process and the considerable filtering which is necessary to identify outstanding targets.

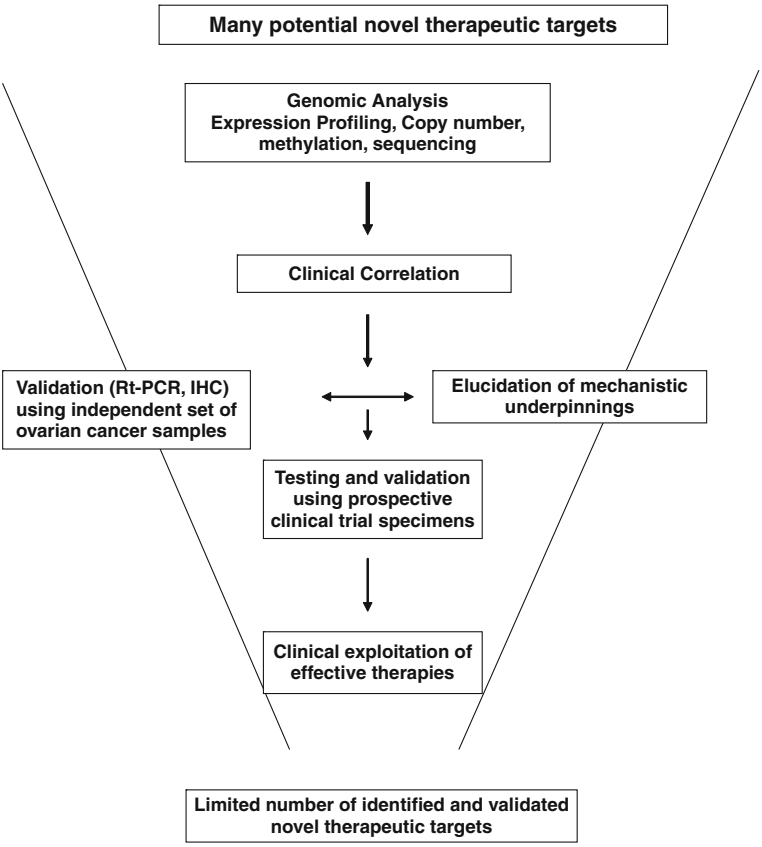

Fig. 2.6 Schematic of screening for novel therapeutic targets

2.9 Conclusion

The recent molecular revolution has provided enormous potential for the better treatment of many human diseases including cancer. The variety of cell surface receptors, signaling pathways, and nuclear proteins that stimulate cellular proliferation or inhibit cell death provides a rich source for the identification of novel therapeutic targets and the subsequent development of clinically relevant molecular agents for the treatment of cancer. In the field of gynecologic oncology, we are now just beginning to investigate new target pathways and agents. Only by utilizing our current and ongoing understanding of the contextual biology that underpins ovarian cancer can we continue to design and use agents that will significantly impact on the mortality from this lethal disease. By combining genomic and bioinformatic analysis, with careful validation and applying high-throughput drug discovery approaches that include cell-based screening programs, it will be possible to discover novel

therapeutic targets and exploit them in rational clinical approaches. These new biologic therapies will usher in a new era of customized therapy that will certainly revolutionize the way we approach gynecologic cancer patients.

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