Review

Cancer Research

The Changing View of High-Grade Serous Ovarian Cancer

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Abstract

The classification of epithelial ovarian cancer has been substantially revised, with an increased appreciation of the cellular origins and molecular aberrations of the different histotypes. Distinct patterns of signaling-pathway disruption are seen between and within histotypes. Large-scale genomic studies of high-grade serous cancer, the most common histotype, have identified novel molecular subtypes that are associated with distinct biology and clinical outcome. High-grade serous cancers are characterized by few driver point mutations but abundant DNA copy number aberrations. Inactivation of genes associated with DNA damage repair underlies responses to platinum and PARP inhibitors. Here we review these recent developments. *Cancer Res; 72(11); 2701–4. ©2012 AACR.*

Introduction

Epithelial ovarian cancer is the fifth most frequent cause of cancer death in women in Western countries, and overall survival rates have improved little in the last 20 to 30 years (1). Serous, endometrioid, clear-cell, and mucinous ovarian cancers are the 4 most common histotypes, and until recently, these cancers were thought to arise from ovarian surface epithelium. Pathological and molecular studies have provided a strikingly different view of epithelial ovarian cancer in which the majority of invasive tumors are now thought to arise from nonovarian tissues. By examining focal premalignant lesions and carcinoma *in situ* in the fallopian tubes of women with germline *BRCA1/2* mutations who were undergoing prophylactic risk-reducing surgery, investigators identified the secretory cells of the distal fallopian tube as the likely progenitor of at least a proportion of high-grade serous cancers [HGSC (2)]. Identical mutations in *TP53* were found in fallopian tube tumors and in deposits throughout the abdomen of women with advanced disease (2), strengthening the link between the fallopian tube and HGSC, and helping to focus attention on the likely progenitor cells. Studies involving DNA damage by irradiation of explants of normal fallopian tubes showed delayed repair of DNA double-strand breaks in the secretory cells compared with adjacent ciliated cells, suggesting that secretory cells are more susceptible to accumulation of mutagenic injury (3).

Previously established epidemiological and pathological associations between endometriosis and endometrioid and clear-cell ovarian cancer were strengthened by the identification of high-frequency somatic mutations in *ARID1a*, a chromatin-remodeling gene, in adjacent endometriotic and cancerous lesions (4). Invasive mucinous ovarian cancers, once considered the second most common histotype, are now thought to account for only 2% to 3% of invasive cancers, with the remainder being metastases to the ovary, most commonly from the gastrointestinal tract (5). Therefore, although the actual contribution of the ovary to the spectrum of epithelial ovarian cancers is still debated, it is now apparent that the histotypes should be considered as distinct diseases originating from different cell types (1).

Tumor grade, a pathological index of cellular aberration, provides additional stratification of both serous and endometrioid cancers. Low-grade serous cancers are thought to arise by the transformation of tumors of borderline malignancy, and activating mutations in members of the RAS pathway (*KRAS*, *BRAF*, and *ErbB2*) are found in the majority of these tumors (6). By contrast, HGSCs rarely have activating *RAS* pathway mutations. Their distinctive molecular characteristics are outlined below. Recent immunohistochemical (7) and gene expression studies showed that endometrioid cancers segregate into low-grade tumors with frequent activating mutations in the WNT-β-catenin pathway (8) and that high-grade endometrioid cancers are indistinguishable from HGSCs (9).

Molecular Characteristics of HGSCs

The reclassification of epithelial ovarian cancer led to the identification of specific molecular events that were obscured when the different histotypes were combined. For example, early estimates of the *TP53* mutation rate in ovarian cancer varied widely (10), in part because many studies aggregated different histotypes. By narrowing our focus to a specific histotype, we were able to show that *TP53* gene mutations occur in almost 100% of HGSCs (11). Germline and somatic mutations in *BRCA1/2* and other members of a pathway that controls homologous recombination repair (HRR) of DNA double-strand breaks, such as *PALB2*, *RAD51*, *RAD50*, *BARD1*, *CHK2*, and *BRIP1* (12), occur much more frequently in HGSCs than in other ovarian cancer histotypes.

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A recent whole-exome analysis performed as part of the Cancer Genome Atlas (TCGA) study of almost 500 HGSCs identified no new common somatic mutations in HGSCs (13) but confirmed that these tumors are dominated by widespread DNA copy number aberrations (14). These and other findings suggest a model in which HGSCs preferentially evolve as a consequence of initial disruption of DNA repair, followed by chromosomal instability, copy number change, and segregation into molecular subtypes (15). Consistent with this notion, a hypermutator phenotype was associated with tumors arising in a germline *BRCA2* mutant background (16).

The frequent occurrence of *BRCA* pathway disruption in HGSCs, clinical responses to agents that target defects in HRR in *BRCA1/2* carriers and noncarriers (17), and gene expression studies of germline *BRCA1/2* mutant and wild-type tumors (18) suggested that most HGSCs may be BRCA-like (19). However, it now seems that only half of HGSCs have identifiable mutations or methylation of BRCA pathway genes (13), which accords well with functional studies involving the formation of γH2Ax-positive repair foci following DNA damage of HGSC cells (20). The molecular features of HGSCs that lack obvious BRCA pathway disruption are still poorly understood, although amplification of the cell-cycle regulator *CCNE1* is predominantly associated with this group (13).

A gene expression analysis of more than 300 HGSCs as part of the Australian Ovarian Cancer Study identified distinct molecular subtypes (21) that have been designated with neutral descriptors (C1, C2, C4, and C5) until the key pathways that control subtype behavior become fully apparent. The 4 molecular subtypes were validated in the TCGA study (13) and are associated with distinct clinical outcomes (22). We recently found that amplification and overexpression of *MYCN* and a downstream pathway involving *LIN28B*, *LET7*, and the chromatin-modifying *HMGA2* are specifically associated with the C5 subtype (22), consistent with the notion that the subtypes may reflect distinct patterns of oncogene activation. The TCGA study also identified frequent disruptions of the RB, PI3K/RAS, NOTCH, and FOXM1 pathways in HGSC, which were independent of molecular subtype (13).

Making sense of the many DNA copy-number changes in HGSCs remains an important goal. The widespread changes in DNA copy number involve a large number of genes at various levels of gain or loss and frequency, such as the 8q24 locus involving *MYC*, which is gained in more than 80% of cases (13). Of interest, a remarkable similarity is seen between the copy-number profiles obtained from different cohorts of HGSC patients, which are different from the pattern seen in clear-cell ovarian cancer (23). The emergence of consistent patterns of chromosomal change in HGSC patient cohorts suggests an underlying interdependency of gains and losses in individual tumors. For example, coamplification of *CCNE1* and the 20q11 locus involving the cell-cycle regulator *TPX2*, among other genes (24), supports the notion of an interaction between certain copy-number events in HGSCs. A systematic RNA interference screen identified a large number of genes, including the transcription factor *PAX8*, as being essential and amplified in ovarian cancer cell lines (25). *PAX8* is strongly expressed in fallopian tube secretory cells and is required for Müllerian system development (26). Protein expression is detectable in almost 100% of HGSC (27). Collectively these findings suggest *PAX8* may be a lineage-specific oncogene, similar to *MITF* in cutaneous melanoma.

Determinants of Response to Therapy and Survival in HGSC

The 5-year survival rate for patients with HGSC is between 35% and 40%, and it is strongly influenced by the extent of disease at presentation (stage) and the amount of residual tumor following primary debulking surgery. However, considerable variation in outcome is observed among HGSC patients matched for stage and debulking status, suggesting that other determinants of survival are at play. Although no molecular predictors of clinical outcome are currently in use (28), several factors are becoming increasingly apparent. Women with a germline mutation in either *BRCA1* or *BRCA2* show higher response rates to chemotherapy, a longer progression-free survival, and improved overall survival compared with noncarriers (13, 16, 29, 30). Improved survival is also seen in women with somatic mutations in *BRCA1/2* but not in those with methylation of the *BRCA1* promoter (13). It seems that the defects in HRR associated with loss of *BRCA* function that create an initial susceptibility to ovarian cancer also render the tumors sensitive to platinum-based DNA crosslinking agents. Intragenic *BRCA1/2* mutations that result in the partial restoration of defective germline alleles were observed in a significant fraction of tumors following platinum-based chemotherapy and the emergence of resistance (31). The apparently strong selective pressure for such germline *BRCA* reversion underscores the importance of *BRCA* dysfunction in influencing platinum sensitivity.

Although most women with HGSC have excellent responses to chemotherapy following primary surgery, 20% to 30% of patients relapse within 6 months of treatment. A supervised analysis of copy-number changes in primary resistant versus responsive HGSC patients identified *CCNE1* amplification as the dominant chromosomal abnormality associated with treatment failure (32). The association of *CCNE1* with poor outcome is lost, however, when the inverse correlation between *CCNE1* amplification and *BRCA* inactivation is taken into account (13). Patients with the C1 molecular subtype of HGSC, which is characterized by a florid reactive stromal response, also commonly fail primary treatment (32). The poor outcome seen in patients with other solid cancers with profound stromal reactions suggests a sharing of mechanisms that influence response to chemotherapy, perhaps associated with poor drug uptake or with cell-adhesion–mediated drug resistance. It is becoming increasingly apparent that HGSC patients who accumulate $CD8^+$ T cells within the epithelial tumor fraction have improved survival compared with those in whom the inflammatory cells are predominantly in the stroma or are few in number, or those who have abundant immune suppressive T-regulatory cells or macrophages (33).

Analysis of paired samples, collected before and after treatment, offers an important opportunity to understand the acquisition of chemotherapy resistance (34). Using this

approach, investigators showed that HDAC4-regulated STAT1 activation (35) and DNA-PK–mediated activation of AKT (36) influence the sensitivity of HGSC cells to platinum, providing potential routes to reverse acquired resistance.

Therapeutic Approaches to HGSC

Although it has proved difficult to advance beyond the use of platinum-paclitaxel–based combination chemotherapy and debulking surgery, which were introduced in the mid 1970s (1), this is likely to change. Numerous clinical trials have investigated the impact of platinum-based chemotherapy, the timing and route of administration, and the scheduling of conventional chemotherapy. A recent Japanese trial showed impressive gains in time-to-relapse and overall survival of HGSC patients following weekly dose-dense administration of paclitaxel/carboplatin (37); however, this strategy has not been shown to be effective in Caucasian populations to date (38).

The central importance of HRR deficiency in HGSCs renders these tumors sensitive to inhibition of other DNA repair proteins, such as PARP. Through inhibition of base excision repair, PARP inhibition forces HRR-deficient cells to use error-prone nonhomologous end joining (39), which was shown to result in selective toxicity to tumor cells and activity in clinical trials of women with germline *BRCA1* or *BRCA2* mutations, as well as in a proportion of noncarriers (17). Important current goals include the identification of patients who are most likely to respond to PARP inhibition and evidence of an improvement in survival over standard regimes (28).

Although molecularly targeted agents are unlikely to replace current therapy soon, several of these agents are showing promise in combination with existing cytotoxics. Attenuation of tumor angiogenesis has been explored in large phase III clinical trials of bevacizumab, an inhibitor antibody directed toward VEGF (ClinicalTrials.gov identifier: GOG-0218, NCT00262847; ICON7, NCT00483782; OCEANS, NCT00434642). The patients required sustained treatment with bevacizumab during and after the end of conventional chemotherapy to achieve a significant increase in time-to-relapse. Current studies suggest that maintenance on bevacizumab until disease progression is required for patients to achieve a substantial benefit, resulting in a substantial increase in the cost of treatment. A lack of effective predictors of response, including whether patients with specific molecular subtypes of HGSC would benefit and a poor understanding of the determinants of resistance, are important limitations to the use of bevacizumab at this time.

Given the clear evidence of CyclinE1's amplicon-dependent cellular addiction (24) as well as its druggability, this protein represents a potentially important therapeutic target in HGSC through inhibition of its association with cyclin-dependent kinases, including cdk2, or targeting of CyclinE1 protein processing. In addition, because *CCNE1* amplification occurs preferentially in HGSC without germline BRCA1/2 mutations, it offers a molecular target in a group of patients who are less likely to benefit from new agents such as PARP inhibitors.

Conclusions

In the last few years, the view of ovarian cancer has changed substantially. Efforts to personalize therapy have focused on elucidating the biology of the specific subtypes. An improved understanding of the progenitors of different histotypes, including HGSC, is facilitating the development of mouse models that recapitulate the human disease (40), which up to now have been elusive. The utility of cell lines for preclinical testing requires well-characterized/annotated lines stratified for subtypes. Comprehensive genomic studies have revealed many of the molecular determinants of the HGSC genome, providing the foundation for a synthesis of the interacting pathways that control the growth and survival of this tumor.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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