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Biomarkers in the Ovary

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Abstract Alterations in specific oncogenes and tumor suppressor genes that serve as surrogate markers of malignant transformation have been identified in ovarian cancers. Overexpression of the HER-2/*neu* oncogene occurs in approximately 30% of breast and ovarian cancers. In most studies, HER-2/*neu* overexpression has correlated with poor survival. Although mutation of the K-*ras* oncogene has been found in some mucinous ovarian cancers, mutations in this gene appear to be more common in borderline ovarian tumors. Amplification of c-*myc* occurs in approximately 30% of ovarian cancers and is more frequently seen in serous cancers. Mutation of the p53 tumor suppressor gene, with resultant overexpression of mutant p53 protein, occurs in 50% of Stage III/IV and 15% of Stage I/II ovarian cancers. Most p53 mutations in ovarian cancers are transitions, which suggests that they arise spontaneously rather than due to exogenous carcinogens. In contrast to the acquired genetic alterations described above that are a feature of sporadic ovarian cancers, a small fraction of epithelial ovarian cancers arise due to inherited genetic defects. Recently, the BRCA1 tumor suppressor gene on chromosome 17q was identified and shown to be responsible for some cases of hereditary breast and ovarian cancer. Families in which mutations in this gene exist are usually characterized by early age of disease onset. Presently, it remains unclear what fraction of hereditary ovarian cancers are due to BRCA1 mutations.

Key words: HER-2/*neu*, oncogenes, ovarian cancer, p53, tumor suppressor genes

The risk of epithelial ovarian cancer is decreased by factors that suppress ovulation (*e.g.*, pregnancy, breast feeding, oral contraceptive pill); however, uninterrupted ovulation (nulliparity) and hyperovulation (infertility drugs) have been associated with increased risk. This suggests that ovulation plays a critical role in ovarian carcinogenesis. There is evidence to suggest that uninterrupted ovulation may facilitate the development of ovarian cancer by increasing exposure to gonadotropins. In addition, the ovulatory defect in the ovarian surface requires proliferation of epithelial cells, which may increase the frequency of spontaneous mutations.

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In the past decade, studies have begun to elucidate the complex sequence of molecular events involved in the pathogenesis of ovarian cancer. As with other cancers, ovarian cancer is thought to arise from sequential damage to oncogenes and tumor suppressor genes normally involved in regulation of cellular proliferation, differentiation, and senescence. Early genetic alterations likely result in a premalignant lesion with secondary changes required for outgrowth of a clinically recognizable cancer. These genetic alterations can be identified using molecular techniques and can serve as surrogate markers of transformation in the ovary.

ONCOGENES

Oncogenes encode proteins that ordinarily participate in growth stimulatory pathways in normal cells. Activation of these genes due to

amplification, translocation, or mutation contributes to the development and maintenance of the transformed phenotype.

Cell membrane receptors that bind peptide growth factors play an important role in transmitting growth stimulatory signals. These receptors are comprised of an extracellular ligand binding domain, a membrane spanning region, and a cytoplasmic tyrosine kinase domain. Our group and others have examined expression of specific peptide growth factor receptors including the epidermal growth factor receptor (EGFR) in ovarian cancers [1]. We found that EGFR was detectable in 77% of advanced stage epithelial ovarian cancers, and survival of patients whose ovarian cancers did not express detectable EGFR was significantly better than that of patients with EGFR-positive cancers. Similarly, Kohler and coworkers [2] found that the 40% of ovarian cancers with the highest EGFR levels had the worst prognosis. Although EGFR expression varies between ovarian cancers, the number of receptors present in ovarian cancers is similar to that seen in normal ovarian epithelial cells [3] and gene amplification has not been noted.

Slamon *et al.* [4] has shown that some human breast cancers express increased levels of the HER-2/*neu* receptor tyrosine kinase, usually due to amplification of the number of copies of the HER-2/*neu* gene. Overexpression of HER-2/*neu* in breast cancer has been associated with poor survival. Slamon *et al.* [4] also found that HER-2/*neu* was overexpressed in one-third of ovarian cancers and increased expression was associated with poor survival. Similarly, we found that 32% of ovarian cancers overexpressed HER-2/*neu* relative to normal ovarian epithelium [5]. Patients whose cancers had normal HER-2/*neu* expression were more likely to achieve a negative second-look laparotomy compared with patients whose cancers overexpressed HER-2/*neu*. Additionally, survival of patients in our study whose cancers overexpressed HER-2/*neu* was strikingly worse than that of patients whose cancers had normal expression. Although some studies have not confirmed the association between HER-2/*neu* overexpression and poor prognosis, increased expression was seen in a fraction of cases, suggesting that this is a useful biomarker associated with transformation [6,7].

The *ras* family of G proteins is also thought to play a critical role in regulating cellular proliferation. It has been shown that G proteins often undergo point mutations in codons 12, 13 or 61 during carcinogenesis, resulting in a constitutively activated molecule. *ras* Mutations do not appear to be a common feature of invasive serous epithelial ovarian cancers [8–10]; however, K-*ras* mutations have been noted more frequently in mucinous ovarian cancers, but these tumors account for a small fraction of epithelial ovarian cancers. In contrast, K-*ras* mutations are common in borderline epithelial ovarian tumors, occurring in 20–50% of cases [11,12]. Thus, studies of the K-*ras* oncogene suggest that the molecular pathology of borderline tumors differs from that of invasive epithelial ovarian cancers. One possible interpretation of this data is that borderline tumors may be a distinct pathologic entity rather than an early stage of invasive cancer.

Proliferation occurring in response to signals generated at the periphery of the cell leads to changes in gene expression and DNA synthesis. In this regard, a family of genes whose products bind DNA and regulate gene transcription has been described. When overexpressed, these transcription factors can act as oncogenes. Among the transcriptional activating factors involved in stimulating proliferation, the *myc* family has most often been implicated in the development of human cancers. In this regard, amplification of the c-*myc* oncogene occurs in some epithelial ovarian cancers. A study analyzing 51 epithelial ovarian cancers found c-*myc* overexpression in 37% of cases [13], and more frequently in advanced stage serous cancers.

TUMOR SUPPRESSOR GENES

Tumor suppressor genes encode proteins that normally restrain proliferation at inappropriate times. Loss of tumor suppressor function usually involves deletion of one copy of the gene, followed by mutation of the second copy.

Loss of p53 tumor suppressor gene function is the most frequent genetic event described thus far in human cancers. Normally, p53 protein inhibits proliferation by binding to transcriptional regulatory elements in DNA. Beyond simply inhibiting proliferation, normal p53 is thought to play an active role in preventing cancer. In this regard, p53 functions as a surveillance mechanism; cells that have undergone genetic damage

are arrested in the G_1 phase of the cell cycle to allow for DNA repair. If DNA repair is inadequate, p53 can trigger programmed cell death (apoptosis).

It has been shown that many cancers have point mutations in one copy of the p53 gene which result in an inactive protein product that cannot bind DNA. As is the case for other tumor suppressor genes, mutation in one copy of the p53 gene is often accompanied by deletion of the second copy, leaving the cancer cell with only mutant p53 protein. While normal cells have low levels of p53 protein because it is rapidly degraded, mutant p53 proteins are resistant to degradation and overaccumulate in the nucleus. This relative overexpression of mutant p53 protein can be detected immunohistochemically.

Our group and others have examined p53 in ovarian cancers. It has been shown that p53 immunostaining is not seen in normal ovaries or benign epithelial ovarian tumors; nuclear staining consistent with overexpression of mutant p53 is seen in approximately 50% of advanced (Stage III/IV) cancers [14], 15% of early (Stage IA/B) cancers [15], and 4% of borderline tumors [16]. In addition, mutations in the p53 gene have been identified in over 90% of ovarian cancers in which immunostaining is seen. The mutations are diverse, but occur in evolutionarily conserved regions of the gene (exons 5–8) that encode functionally important parts of the molecule [14,15,17,18]. Amino acid changes in these critical regions lead to subtle alterations in the structure of the protein that prevent it from suppressing tumorigenesis. The majority of these mutations are transitions [17,18], suggesting that p53 mutations in ovarian cancers arise due to spontaneously occurring DNA damage that accumulates with aging.

Linkage analysis of several large familial breast/ovarian cancer kindreds initially suggested that this disease was caused by a gene residing on chromosome 17q21. Recently, this putative tumor suppressor gene (BRCA1) has been identified, and germline mutations in BRCA1 have been shown to segregate with the disease in breast/ovarian cancer families [19,20]. Presumably, affected individuals in these kindreds inherit one mutant copy of BRCA1, with tumor development dependent on subsequent loss of the second copy of the gene in a single cell. In this regard, allelic deletion in the region of chromosome 17q that includes BRCA1 frequently occurs in familial breast-ovarian cancer cases. Although familial breast/ovarian cancer is relatively rare because loss of heterozygosity on 17q also frequently occurs in sporadic breast and ovarian cancers, BRCA1 might also be involved in the development of a fraction of these more common cancers.

Initial analysis of sporadic breast and ovarian cancers found an 11% (4/44) incidence of BRCA1 mutations [19], including 3/32 breast cancers and 1/12 ovarian cancers. BRCA1 mutations in all four of these cases were found both in the cancer and the germline, indicating an inherited defect. In addition, all four of these patients were under 45 years of age at diagnosis. BRCA1 mutations were seen only in women with early onset cancers; although only 15–20% of epithelial ovarian cancers occur before age 50, germline mutations in BRCA1 may be responsible for a significant fraction of these cases.

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