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COMMENTARY

Human Ovarian Cancer of the Surface Epithelium

Andrew Berchuck and Michael Carney*

DIVISION OF GYNECOLOGIC ONCOLOGY, DUKE UNIVERSITY MEDICAL CENTER, DURHAM, NC 27710, U.S.A.

ABSTRACT. Epidemiologic studies have shown that the risk of cancer in the ovarian surface epithelium is decreased by factors that suppress ovulation, whereas uninterrupted ovulation has been associated with increased risk. This suggests that ovulation may play a critical role in ovarian carcinogenesis. More recently, molecular studies have demonstrated alterations in specific oncogenes and tumor suppressor genes in ovarian cancers. Overexpression of the HER-2/*neu* oncogene occurs in approximately 30% of ovarian cancers and correlates 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, 5–10% of ovarian cancers probably arise due to inherited genetic defects. Recently, the *BRCA1* tumor suppressor gene has been identified and shown to be responsible for most cases of hereditary ovarian cancer. Further studies are needed to augment our understanding of the molecular pathogenesis of ovarian cancer. BIOCHEM PHARMACOL **54**;5:541–544, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Ovarian cancer; ovary; oncogene; tumor suppressor gene; *p53* gene

Epidemiologic studies have shown consistently that the risk of epithelial ovarian cancer is decreased by factors that suppress ovulation (pregnancy, breast-feeding, oral contraceptive pill). For example, in an analysis of 12 case-control studies of ovarian cancer, the protective effect of a single term pregnancy was estimated to be 40% [1]. In addition, use of oral contraceptive pills for 5 years decreases ovarian cancer risk by approximately 50%. Further support for the causative role of ovulation comes from the observation that cancer of the ovarian surface epithelium is rare in animal species that ovulate infrequently, whereas it is common in hens, which, like humans, are frequent ovulators [2]. Finally, epithelial ovarian cancer is exceedingly rare in women with Turner's syndrome, who are anovulatory.

Several causative mechanisms have been suggested to explain the relationship between uninterrupted ovulation and the development of ovarian cancer. These theories all are supported by some experimental data and likely represent complementary rather than competing hypotheses. First, it has been suggested that ovulation may increase the risk of ovarian cancer because of exposure to the epithelium to high levels of steroid hormones and gonadotropins. Second, the ovulatory defect in the ovarian surface likely results in proliferation of epithelial cells, which may increase the frequency and accumulation of spontaneous mutations. Finally, it has been suggested that ovulation may lead to entrapment of epithelial cells in the underlying stroma with subsequent formation of inclusion cysts. These inclusion cysts could represent precursor lesions in which transformation is facilitated by the presence of hormones and/or growth factors in the cyst fluid.

In the past decade, our group and others have begun to elucidate the complex sequence of molecular events involved in the pathogenesis of ovarian cancer. As is the case for other cancers, it is thought that ovarian cancer arises due to sequential damage to oncogenes and tumor suppressor genes, which normally are involved in the regulation of cellular proliferation, differentiation, and senescence. Early genetic alterations likely result in a pre-malignant lesion with secondary changes required for the outgrowth of a clinically recognizable cancer.

GROWTH STIMULATORY PATHWAYS

Oncogenes encode proteins that participate in growth stimulatory pathways in normal cells. Activation of these genes due to amplification, translocation, or mutation has been shown to contribute to the development of many types of human cancers.

Cell membrane receptors that bind peptide growth are one class of proto-oncogene products that 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

* Corresponding author: Dr. Andrew Berchuck, Division of Gynecologic Oncology, Duke University Medical Center, Box 3079, Durham, NC 27710. Tel. (919) 684-3765; FAX (919) 684-8719.

tyrosine kinase domain. Our group and others have examined expression of specific peptide growth factor receptors including the EGF† receptor in ovarian cancers [3]. We found that EGF receptor was detectable in 77% of advanced stage epithelial ovarian cancers, and survival of patients whose ovarian cancers did not express detectable EGF receptor was significantly better than that of patients with EGF receptor positive cancers. Similarly, Kohler and co-workers [4] found that the 40% of ovarian cancers with the highest EGF receptor levels had the worst prognosis. Although EGF receptor expression varies between ovarian cancers, the number of receptors present in ovarian cancers is similar to that seen in normal ovarian epithelial cells [5], and gene amplification has not been noted.

Slamon *et al.* [6] have 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.* [6] 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 [7]. 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*. In addition, in our study, survival of patients whose cancers overexpressed HER-2/*neu* was strikingly worse than that of patients whose cancers had normal expression. Not all studies have confirmed the association between HER-2/*neu* overexpression and poor prognosis in ovarian cancer, however [8, 9].

The *ras* family of G proteins also are thought to play a critical role in regulation of cellular proliferation. It has been shown that G proteins often undergo point mutations in codons 12, 13, or 61 during the process of carcinogenesis, which result in a constitutively activated molecule. *ras* mutations do not appear to be a common feature of invasive serous epithelial ovarian cancers, however [10–12]. 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 [13, 14]. 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 these data is that borderline tumors may be a distinct pathologic entity rather than an early stage in the development of invasive cancers.

If proliferation is to occur in response to signals generated at the periphery of the cell, these events must lead to changes in gene expression and DNA synthesis. In this regard, a family of genes whose products bind to 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. In a study in which 51 epithelial ovarian cancers were analyzed, c-*myc* overexpression was observed in 37% of cases [15] and was more frequent in advanced stage serous cancers.

GROWTH INHIBITORY PATHWAYS

Unlike peptide growth factors that stimulate proliferation, the TGF-β family of growth factors inhibit proliferation. Three closely related forms of TGF-β have been discovered that are encoded by separate genes (TGF-β1, TGF-β2, and TGF-β3). TGF-β is secreted from cells in an inactive form bound to a portion of its precursor molecule from which it must be cleaved to release biologically active TGF-β. TGF-β interacts with cell surface receptor serine/threonine kinases. Our group and others have shown that while normal ovarian epithelial cells produce, activate, and are growth inhibited by TGF-β [16], most ovarian cancer cell lines have lost the ability to either produce, activate, or respond to TGF-β [16–21]. These data suggest that TGF-β might normally act as an autocrine growth inhibitory factor in normal ovarian epithelium, which under most circumstances is thought to have a relatively low growth rate. Conversely, since most immortalized ovarian cancer cell lines were found to lack an intact TGF-β autocrine loop, loss of this growth inhibitory pathway might play a role in the development of some ovarian cancers.

Although convenient to work with, immortalized cell lines frequently have undergone profound genetic alterations following establishment and multiple passages in tissue culture. In practice, less than 5% of primary ovarian cancers that are grown in tissue culture subsequently become immortalized cell lines while the vast majority stop dividing after several weeks. Because immortalized ovarian cancer cell lines represent the exception rather than the norm with regard to growth, they are poor models in which to study growth regulation. More recently, we found that monolayer cultures of 19 of 20 primary ovarian cancer cells were sensitive to the growth inhibitory effect of TGF-β [22]. Similarly, Daniels *et al.* found that colony formation of 7 of 9 primary ovarian cancers in soft agar was inhibited by TGF-β. Although we found that some ovarian cells did not produce immunohistochemically detectable TGF-β, since this growth inhibitor is present in ascites, production by the ovarian cancer cells themselves may not be necessary to activate this inhibitory pathway *in vivo*. The biological significance of growth inhibition by members of the TGF-β family remains uncertain, but it appears likely that the rate of cellular proliferation is dependent upon the net action of growth-stimulating and growth-inhibiting factors.

† *Abbreviations:* EGF, epidermal growth factor; and TGF-β, transforming growth factor-β.

Tumor-suppressor genes encode proteins that normally act to restrain proliferation at times when it is inappropriate. Most tumor suppressor gene products encode nuclear proteins involved in cell cycle regulation. Loss of tumor suppressor function usually involves deletion of one copy of the gene followed by mutation of the second copy of the gene. Most hereditary cancers arise due to inheritance of a mutant copy of one of the tumor suppressor genes.

Loss of *p53* tumor-suppressor gene function is the most frequent genetic event prescribed 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 in which 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, also known as 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 to DNA. As is the case for other tumor suppressor genes, mutation of one copy of the *p53* gene often is accompanied by deletion of the other copy, leaving the cancer cell with only mutant p53 protein. While normal cells have low levels of p53 protein because it is degraded rapidly, 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, whereas nuclear staining consistent with overexpression of mutant p53 is seen in approximately 50% of advanced stage cancers [24], 15% of early stage cancers [25] and 4% of borderline tumors [26]. 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 [24, 25, 27, 28]. Amino acid changes in these critical regions lead to subtle alterations in the structure of the protein that prevent it from suppressing tumorigenesis. The finding that the majority of these mutations are transitions [27, 28] suggests that p53 mutations in ovarian cancers arise spontaneously during proliferation that occurs to repair surface defects associated with ovulation.

HEREDITARY OVARIAN CANCER

Approximately 5–10% of epithelial ovarian cancers are thought to be due to inherited mutations in cancer susceptibility genes. Recently, the *BRCA1* gene on chromosome 17q has been identified, and this gene is thought to be responsible for the majority of cases of hereditary ovarian cancer [29–31]. The lifetime risk of developing breast and/or ovarian cancer is 90% in mutation carriers. *BRCA1* likely is a tumor suppressor gene, but the molecular function of the BRCA1 protein remains unknown. Presumably, however, affected individuals inherit one mutant copy of *BRCA1*, with tumor development dependent on subsequent loss of the second copy of the gene in a single cell. Although allelic deletion in the region of chromosome 17q that includes *BRCA1* frequently occurs in sporadic ovarian cancer cases, acquired mutations in *BRCA1* appear to be relatively rare [29, 32].

With the ability to identify inherited mutations in *BRCA1*, prophylactic oophorectomy and other interventions intended to decrease cancer mortality can be offered to women who carry a mutation; but the optimal strategy for decreasing cancer mortality in *BRCA1* families has not yet been determined. To facilitate further clinical and basic research in this field, our group and others have established multidisciplinary hereditary breast/ovarian cancer clinics that offer a wide range of services including *BRCA1* testing, genetic counseling, and cancer prevention and treatment.

CONCLUSION

It has been appreciated for some time that epithelial ovarian cancer is a heterogeneous disease with respect to etiologic risk factors, histologic features, patterns of spread, response to therapy, and survival. Now, molecular studies are leading us towards an understanding of the underlying genetic alterations that are the basis of this heterogeneity. In addition, these studies have the potential to elucidate the temporal sequence of events that occur prior to the development of a clinically recognizable ovarian cancer. As our knowledge of the molecular genetics of ovarian cancer matures, hopefully this will be translated into improvements in early diagnosis, treatment, and prevention.

References

1. Whittemore AS, Harris R, Itnyre J and the Collaborative Ovarian Cancer Group, Characteristics relating to ovarian cancer risk. Collaborative analysis of twelve US case-control studies. II. Invasive epithelial ovarian cancers in white women. *Am J Epidemiol* **136:** 1184–1203, 1992.
2. Fredrickson TN, Ovarian tumors of the hen. *Environ Health Perspect* **73:** 35–51, 1987.
3. Berchuck A, Rodriguez GC, Kamel A, Dodge RK, Soper JT, Clarke-Pearson DL and Bast RC Jr, Epidermal growth factor receptor expression in normal ovarian epithelium and ovarian cancer. I. Correlation of receptor expression with prognostic factors in patients with ovarian cancer. *Am J Obstet Gynecol* **164:** 669–674, 1991.
4. Kohler M, Janz I, Wintzer HO, Wagner E and Bauknecht T, The expression of EGF receptors, EGF-like factors and c-*myc* in ovarian and cervical carcinomas and their potential clinical significance. *Anticancer Res* **9:** 1537–1547, 1989.
5. Rodriguez GC, Berchuck A, Whitaker RS, Schlossman D, Clarke-Pearson DL and Bast RC Jr, Epidermal growth factor receptor expression in normal ovarian epithelium and ovarian cancer. II. Relationship between receptor expression and

response to epidermal growth factor. *Am J Obstet Gynecol* **164:** 745–750, 1991.
6. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin LJ, Stuart SG, Udove J, Ullrich A and Press MF, Studies of HER-2/*neu* proto-oncogene in human breast and ovarian cancer. *Science* **244:** 707–712, 1989.
7. Berchuck A, Kamel A, Whitaker R, Kerns B, Olt G, Kinney R, Soper JT, Dodge R, Clarke-Pearson DL, Marks S, McKenzie S, Yin S and Bast RC Jr, Overexpression of HER-2/*neu* is associated with poor survival in advanced epithelial ovarian cancer. *Cancer Res* **50:** 4087–4091, 1990.
8. Rubin SC, Finstad CL, Wong GY, Almadrones L, Plante M and Lloyd KO, Prognostic significance of HER-2/*neu* expression in advanced ovarian cancer. *Am J Obstet Gynecol* **168:** 162–169, 1993.
9. Kacinski BM, Mayer AG, King BL, Carter D and Chambers S, *Neu* protein overexpression in benign, borderline, and malignant ovarian neoplasms. *Gynecol Oncol* **44:** 245–253, 1992.
10. Enomoto T, Inoue M, Perantoni AO, Terakawa N, Tanizawa O and Rice JM, K-*ras* activation in neoplasms of the human female reproductive tract. *Cancer Res* **50:** 6139–6145, 1990.
11. Feig LA, Bast RC Jr, Knapp RC and Cooper GM, Somatic activation of ras^K gene in a human ovarian carcinoma. *Science* **223:** 698–701, 1984.
12. Haas M, Isakov J and Howell SB, Evidence against *ras* activation in human ovarian carcinomas. *Mol Biol Med* **4:** 265–275, 1987.
13. Teneriello MG, Ebina M, Linnoila RI, Henry M, Nash JD, Park RC and Birrer MJ, *p53* and Ki-*ras* gene mutations in epithelial ovarian neoplasms. *Cancer Res* **53:** 3103–3108, 1993.
14. Mok SC-H, Bell DA, Knapp RC, Fishbaugh PM, Welch WR, Muto MG, Berkowitz RS and Tsao S-W, Mutation of K-*ras* protooncogene in human ovarian epithelial tumors of borderline malignancy. *Cancer Res* **53:** 1489–1492, 1993.
15. Tashiro H, Niyazaki K, Okamura H, Iwai A and Fukumoto M, c-*myc* overexpression in human primary ovarian tumors: Its relevance to tumor progression, *Int J Cancer* **50:** 828–833, 1992.
16. Berchuck A, Rodriguez GC, Olt GJ, Boente MP, Whitaker RS, Arrick B, Clarke-Pearson DL and Bast RC Jr, Regulation of growth of normal ovarian epithelial cells and ovarian cancer cell lines by transforming growth factor-β. *Am J Obstet Gynecol* **166:** 676–684, 1992.
17. Berchuck A, Olt GJ, Everitt L, Soisson AP, Bast RC Jr and Boyer CM, The role of peptide growth factors in epithelial ovarian cancer. *Obstet Gynecol* **75:** 255–262, 1990.
18. Marth C, Lang T, Koza A, Mayer I and Daxenbichler G, Transforming growth factor-beta and ovarian carcinoma cells: Regulation of proliferation and surface antigen expression. *Cancer Lett* **51:** 221–225, 1990.
19. Bartlett JMS, Rabiasz GJ, Scott WN, Langdon SP, Smyth JF and Miller WR, Transforming growth factor-β mRNA expression in growth control of human ovarian carcinoma cells. *Br J Cancer* **65:** 655–660, 1992.
20. Jozan S, Guerrin M, Mazars P, Dutaur M, Monsarrat B, Cheutin F, Bugat R, Martel P and Valette A, Transforming growth factor β1 (TGF-β1) inhibits growth of a human ovarian cancer cell line (OVCCR1) and is expressed in human ovarian tumors. *Int J Cancer* **52:** 766–770, 1992.
21. Zhou L and Leung BS, Growth regulation of ovarian cancer cells by epidermal growth factor and transforming growth factors α and β1. *Biochim Biophys Acta* **1180:** 130–136, 1992.
22. Hurteau J, Rodriguez GC, Whitaker RS, Shain S, Bast RC Jr and Berchuck A, Effect of transforming growth factor-β on proliferation of human ovarian cancer cells obtained from ascites. *Cancer* **74:** 93–99, 1994.
23. Daniels AM, McPherson JM, Daniels JR and Piez KA, Unusual antiproliferative effects of transforming growth factors-β1 and β2 against primary cells from human tumors. *Biotherapy* **1:** 133–137, 1989.
24. Marks JR, Davidoff AM, Kerns BJ, Humphrey PA, Pence JC, Dodge RK, Clarke-Pearson DL, Iglehart JD, Bast RC Jr and Berchuck A, Overexpression and mutation of p53 in epithelial ovarian cancer. *Cancer Res* **51:** 2979–2984, 1991.
25. Kohler MF, Kerns B-J, Humphrey PA, Marks JR, Bast RC Jr, and Berchuck A, Mutation and overexpression of p53 in early-stage epithelial ovarian cancer. *Obstet Gynecol* **81:** 643–650, 1993.
26. Berchuck A, Kohler MF, Hopkins MP, Humphrey PA, Robboy SJ, Rodriguez GC, Soper JT, Clarke-Pearson DL and Bast RC, Overexpression of p53 is not a feature of benign and early-stage borderline epithelial ovarian tumors. *Gynecol Oncol* **52:** 232–236, 1994.
27. Kohler MF, Marks JR, Wiseman RW, Jacobs IJ, Davidoff AM, Clarke-Pearson DL, Soper JT, Bast RC Jr and Berchuck A, Spectrum of mutation and frequency of allelic deletion of the p53 gene in ovarian cancer. *J Natl Cancer Inst* **85:** 1513–1519, 1993.
28. Kupryjańczyk J, Thor AD, Beauchamp R, Merritt V, Edgerton SM, Bell DA, and Yandell DW, p53 gene mutations and protein accumulation in human ovarian cancer. *Proc Natl Acad Sci USA* **90:** 4961–4965, 1993.
29. Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y, Eddington K, McClure M, Frye C, Weaver-Feldhaus J, Ding W, Gholami Z, Söderkvist P, Terry L, Jhanwar S, Berchuck A, Iglehart JD, Marks J, Ballinger DG, Barnett JC, Skolnick MH, Kamb A and Wiseman R, *BRCA1* mutations in primary breast and ovarian carcinomas. *Science* **266:** 120–122, 1994.
30. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A and Skolnick MH, A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science* **266:** 66–71, 1994.
31. Shattuck-Eidens D, McClure M, Simard J, Labrie F, Narod S, Couch F, Hoskins K, Weber B, Castilla L, Erdos M, Brody L, Friedman L, Ostermeyer E, Szabo C, King M-C, Jhanwar S, Offit K, Norton L, Gilewski T, Lubin M, Osborne M, Black D, Boyd M, Steel M, Ingles S, Haile R, Lindblom A, Olsson H, Borg A, Bishop DT, Solomon E, Radice P, Spatti G, Gayther S, Ponder B, Warren W, Stratton M, Liu Q, Fujimura F, Lewis C, Skolnick M and Goldgar DE, A collaborative survey of 80 mutations in the *BRCA1* breast and ovarian cancer susceptibility gene. Implications for presymptomatic testing and screening. *JAMA* **273:** 535–541, 1995.
32. Takahashi H, Behbakht K, McGovern PE, Chiu H-C, Couch FJ, Weber BL, Friedman LS, King M-C, Furusato M, LiVolsi VA, Menzin AW, Liu PC, Benjamin I, Morgan MA, King SA, Rebane A, Cardonick A, Mikuta JJ, Rubin SC and Boyd J, Mutation analysis of the *BRCA1* gene in ovarian cancers. *Cancer Res* **55:** 2998–3002, 1995.