

Lifestyle and Reproductive Factors and Ovarian Cancer Risk by p53 and MAPK Expression

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Abstract

Background: One model of ovarian cancer development model divides tumors into two types. Type I tumors are characterized by *KRAS* and *BRAF* mutations, which can activate mitogen-activated protein kinase (MAPK). Type II tumors are characterized by tubal precursor lesions with p53 mutations. We evaluated the association between lifestyle and reproductive factors and risk of ovarian cancer defined by p53 and MAPK expression.

Methods: Epithelial ovarian cancer cases ($n = 274$) and controls ($n = 1,907$) were identified from the Nurses' Health Study and Nurses' Health Study II prospective cohorts, and the population-based New England Case-Control study. Reproductive and lifestyle exposures were assessed by questionnaire/interview. We performed immunohistochemical assays for p53 and MAPK expression. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using polytomous logistic regression.

Results: Parity was associated with a decreased risk of p53 wild-type tumors (OR = 0.31; 95% CI, 0.18–0.55), but not p53-mutant

tumors (OR = 0.92; 95% CI, 0.54–1.59) ($P_{\text{heterogeneity}} < 0.01$). Family history of breast or ovarian cancer was associated with risk of MAPK-negative (OR = 2.06; 95% CI, 1.39–3.06), but not MAPK-positive tumors (OR = 0.74; 95% CI, 0.43–1.27; $P_{\text{heterogeneity}} < 0.01$). In cross-classified analyses, family history of breast or ovarian cancer was most strongly associated with p53-mutant/MAPK-negative tumors (OR = 2.33; 95% CI, 1.44–3.75). Differences by MAPK expression were also observed for estrogen plus progesterone hormone therapy use ($P_{\text{heterogeneity}} = 0.03$).

Conclusions: These findings provide evidence that parity, family history, and estrogen plus progesterone hormone therapy use may be differentially associated with tumor subtypes defined by p53 and MAPK expression.

Impact: In future studies, other immunohistochemical markers or gene expression profiles that more clearly define these subtypes should be considered. *Cancer Epidemiol Biomarkers Prev*; 27(1); 96–102. ©2017 AACR.

Introduction

One recent model of ovarian cancer development divides ovarian cancer into two types, integrating histopathology and molecular characteristics (1, 2). Type I tumors are slow growing and include predominantly low-grade serous (LGSC), low-grade endometrioid, clear cell, and mucinous carcinomas (2). Type II tumors are fast growing and include primarily high-grade serous

carcinoma (HGSC). Within these two groups, tumors can be further divided by their potential tissue of origin (e.g., endometrium, fallopian tube, ovary), highlighting the substantial heterogeneity of ovarian cancer (3). Some risk factors for ovarian cancer have been shown to vary by histologic subtype, suggesting that tumors that arise from these two distinct pathways may have different risk profiles (4–6). Tumor protein expression as measured with tissue microarrays (TMAs) may provide additional information to help differentiate etiologic pathways characterizing specific subtypes of ovarian tumors.

While type II tumors are characterized by p53 mutations, type I tumors are characterized by *KRAS*, *BRAF*, and *PTEN* mutations (1, 2). Thus, mitogen-activated protein kinase (MAPK), which is upregulated by *KRAS* and *BRAF*, is hypothesized to act as marker for type I tumors, and aberrant expression of p53 (either no expression or expression in nearly all cells; ref. 7) may act as a marker of type II tumors. The purpose of this study was to evaluate the association between selected lifestyle and reproductive risk factors and risk of ovarian cancer defined by expression of p53 and MAPK in ovarian tumors from the Nurses' Health Study (NHS), Nurses' Health Study II (NHS II), and the New England Case-Control (NECC) study of ovarian cancer.

Materials and Methods

Study populations

The prospective NHS was established in 1976 among 121,700 female registered nurses ages 30 to 55 years from 11 U.S. states. The NHS II was established in 1989 among 116,429 female

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registered nurses ages 25 to 42 years from 14 U.S. states. In both cohorts, women completed biennial questionnaires with detailed information on demographic, lifestyle, and reproductive factors, and medical history since baseline. We conducted our analyses among cases (diagnosed from 1976–2006 for NHS and 1989–2005 for NHS II) and controls from ovarian cancer case–control studies nested within the cohorts on whom we were able to obtain tumor tissue. Incident cases of epithelial ovarian cancer were identified by biennial questionnaire, or, for deceased participants, from linkage to National Death Index (8), postal service, or family members. More detailed information on the identification and confirmation of ovarian cancer cases has been described previously (9). Completion and return of the self-administered questionnaires was treated as informed consent of study participants. The NHS and NHS II were approved by the Institutional Review Board of Brigham and Women's Hospital.

The NECC study is a population-based case–control study of ovarian cancer that was conducted in eastern Massachusetts and New Hampshire in three enrollment phases (phase I, 1992–1997; phase II, 1998–2002; and phase III, 2003–2008). For these analyses, only cases and controls from phase III were included. Cases were identified through tumor registries of area hospitals and controls through town books. In the third phase, 68.3% of eligible cases and 51.2% of eligible controls were enrolled. Controls were frequency matched to cases on age and state of residence. Further details regarding case and control enrollment are described elsewhere (10). Each participant provided written informed consent. The NECC study was approved by the Institutional Review Boards of Brigham and Women's Hospital and Dartmouth Medical School.

Ovarian tumor block collection

In the NHS, 1,183 confirmed epithelial cases diagnosed through 2006 and 201 NHS II confirmed epithelial cases diagnosed through 2005 were screened for tumor block collection eligibility (confirmed cases with a pathology report and surgery). Seven hundred ninety-three (73%) NHS and 124 (62%) NHS II cases were eligible for tumor collection. Paraffin-embedded tissue blocks were received for 314 NHS and 59 NHS II cases and appropriate tissue for TMAs was obtained for 217 NHS and 46 NHS II cases, 18% and 23% of confirmed cases, respectively. After excluding borderline tumors, the final analytic sample for this analysis was 170 and 32 invasive cases, respectively. In the NHS and NHS II, up to four controls ($n = 1050$) with no prior bilateral oophorectomy or menopause due to irradiation, no prior diagnosis of cancer (except nonmelanoma skin cancer) and who were alive at the time of case diagnosis were selected, matching on year of birth.

In the NECC, 213 cases were eligible for tumor block collection (confirmed cases with an invasive tumor, no neoadjuvant chemotherapy, and surgery at Brigham and Women's Hospital, Boston, MA). Tumor blocks were unable to be obtained for 71 cases, were not requested for 39 cases, an additional 25 cases were not included in the current TMA, resulting in the inclusion of 78 cases on the TMA (37% of eligible cases). Subsequently, six borderline tumors were not included this analysis resulting in a final number of eligible invasive cases included from NECC of 72. In NECC, all controls recruited from 2003 to 2008 ($n = 857$) were included.

As previously reported in Shafrir and colleagues ovarian cancer cases from which tumor blocks were obtained were generally similar to cases from which tumor blocks were not obtained with respect to risk factors and tumor characteristics for all included studies (NHS, NHS II, and NECC). For example, 90%, 70%, and 69% of all cases in the NHS, NHS II, and NECC were parous compared with 93%, 76%, and 72% of cases included on the TMA, respectively (11). The major reasons tissue blocks were not obtained included: (i) blocks had been destroyed, (ii) the case was deceased, and (iii) budget constraints. More details about ovarian tumor block collection, including a flow chart with case exclusions details, have been described previously by Shafrir and colleagues (11).

TMA construction and immunohistochemistry

For each case, a primary tumor block was selected, and a study pathologist (J.L. Hecht and M. Gupta) confirmed histology and behavior and assessed grade (I, II, III; refs. 12, 13), circled the area of tumor on the slide, and sent the block/slide to the Dana-Farber/Harvard Cancer Center Specialized Histopathology Services Core for TMA construction. Up to three cores per case were extracted using 0.6 mm (NHS/NHS II) or 1 mm (NECC) diameter hollow needles. Cores were transferred to a recipient paraffin-embedded block and sections were cut to create array slides, which were processed and stained. Details of the immunohistochemistry process have been described previously (14). Briefly, slides were soaked in Xylene overnight, deparaffinized, and antigens were retrieved and stained with the primary antibodies: p53 (mouse monoclonal; clone DO-1; Beckman Coulter; dilution 1:500) and pERK (rabbit monoclonal; p-ERK; Cell Signaling Technologies; dilution 1:150). pERK staining was used as a surrogate for activation of the MAP kinase pathway and tumors with pERK staining were designated MAPK⁺. A gynecologic pathologist (J.L. Hecht) assessed the proportion of reactive versus total cells (0%, 1%–10%, 11%–50%, 51%–90%, >90%). The cores for each case were scored independently. The maximum score of the three cores for each case was used in analyses (14). Cores were designated as not interpretable if tissue was missing from the slide or only a few tumor cell clusters (<20 cells) were present.

Exposure and covariate assessment

The NHS and NHS II baseline and biennial questionnaires collected exposure and covariate information. For this analysis, information was taken from the questionnaire cycle prior to case diagnosis for both the case and the matched control. All NECC participants were interviewed at the time of enrollment and to avoid the possible impact of preclinical disease on exposure status, cases were asked about exposures that occurred at least one year before diagnosis. Controls were asked about exposures that occurred more than one year before the interview date. For all studies (NHS, NHS II, and NECC) information was collected on: age at diagnosis, age at menarche, oral contraceptive use, tubal ligation, parity, menopausal status, age at menopause, postmenopausal hormone therapy (HT) use, and family history of breast or ovarian cancer. Calculation of age at natural menopause excluded women reporting a hysterectomy before menopause.

Statistical analysis

MAPK status was considered positive if >10% of the cells stained positive based on the maximum score of the three cores; p53 was classified as mutant if >50% of the cells or 0% of the cells

stained positive based on the maximum score of the three cores (7, 15, 16). Cases were excluded from analyses if all three cores for that case had only nontumor tissue or noninterpretable MAPK and p53 stains ($n = 13$ NHS, $n = 2$ NHS II, $n = 1$ NECC), leaving 204 (NHS/NHS II) and 44 (NECC) cases for the analyses.

Polytomous logistic regression (17) was used to simultaneously estimate separate odds ratios (ORs) and 95% confidence intervals (CIs) for tumors staining positive and negative for MAPK as well as for p53-mutant and p53 wild-type tumors. Analyses were also conducted defining tumors by joint p53/MAPK status (p53 wild-type/MAPK-, p53 mutant/MAPK-, p53 wild-type/MAPK⁺, p53 mutant/MAPK⁺). For each exposure, we assessed the statistical heterogeneity in the ORs by MAPK or p53 status using a likelihood ratio test comparing a model where the association between the exposure of interest and ovarian cancer risk was allowed to vary by MAPK or p53 expression to another model where the association was not allowed to vary (18). Multivariable models were adjusted for age at diagnosis, cohort, family history of breast or ovarian cancer, oral contraceptive use, parity, menopausal status, and postmenopausal HT use.

To determine whether differential risk factor associations by p53 and MAPK status were explained by tumor histology, we used unconditional logistic regression models in case–case analyses, where tumors mutant/positive for marker expression were considered "cases" and tumors wild-type/negative for expression were considered "controls." We controlled for the covariates above (model one), then added histology (serous, endometrioid, clear cell, mucinous, other) and grade (borderline, I, II, III, unknown; model 2), and calculated the proportion of mediation due to histology and grade on the association between the exposure of interest and p53 or MAPK expression (19, 20). The mediation proportion is defined as the proportion of excess mutant/positive cases relative to wild-type/negative cases that can be attributed to histology and grade. We estimated the proportion of mediation (on the log odds scale) due to histology and grade on the association between the exposure of interest and p53 or MAPK expression using the following equation: $1 - (\ln OR_{\text{adjusted}} / \ln OR_{\text{unadjusted}})$. All P values were based on two-sided tests and were considered statistically significant if $P < 0.05$. Statistical analyses were performed using SAS Version 9.4 (SAS Institute Inc) and Stata 12.1 (StataCorp).

Results

The combined analyses included 274 cases (NHS = 170, NHS II = 32, NECC = 72) and 1,907 controls (NHS = 867, NHS II = 184, NECC = 857). Controls were slightly younger than cases, were more likely to have ever used oral contraceptives, and to have had a tubal ligation (Table 1). Sixty-eight percent of tumors were characterized as p53 mutant ($N = 186$) and 48% were positive for MAPK ($N = 132$). Cases with p53-mutant tumors were older than those with p53 wild-type tumors. p53-mutant tumors were more likely to be high-grade serous (72%) compared with p53 wild-type tumors (40%; Table 1).

The association of lifestyle and reproductive factors with ovarian cancer risk did not significantly differ by p53 or MAPK expression for most of the examined factors. For p53, only the association with parity had significant heterogeneity by p53 expression. Compared with nulliparous women, parous women had a highly significant 69% decreased risk of p53 wild-type tumors (OR = 0.31; 95% CI = 0.18–0.55) and a nonsignificant

8% decreased risk of p53-mutant tumors (OR = 0.92; 95% CI, 0.54–1.59; $P_{\text{heterogeneity}} < 0.01$). Histology and grade explained 29% of the differential association observed; the percentage of the association that was mediated by histology and grade was borderline statistically significant ($P_{\text{mediation}} = 0.06$). No significant heterogeneity by p53 expression was observed for family history of breast or ovarian cancer, oral contraceptive use, tubal ligation, age at menarche, menopausal status, age at menopause, or postmenopausal HT use.

Significant heterogeneity was observed between MAPK-positive and MAPK-negative tumors for family history of breast or ovarian cancer and estrogen plus progesterone HT use (Table 2). A family history of breast or ovarian cancer was associated with an 106% increased risk of MAPK-negative tumors (95% CI, 1.39–3.06) compared with a nonsignificant decreased risk of MAPK-positive tumors (OR = 0.74; 95% CI, 0.43–1.27; $P_{\text{heterogeneity}} < 0.01$). No mediation by histology and grade was detected for this difference in associations. Estrogen plus progesterone HT use was inversely associated with tumors negative for MAPK expression (OR = 0.72; 95% CI, 0.43–1.22) and positively associated with tumors positive for MAPK expression (OR = 1.52; 95% CI = 0.96–2.38; $P_{\text{heterogeneity}} = 0.03$); however, neither effect estimate was statistically significant. Further, no mediation by histology and grade was observed. While not statistically significant ($P_{\text{heterogeneity}} = 0.06$), tubal ligation was more strongly associated with tumors positive for MAPK expression (OR = 0.31; 95% CI, 0.16–0.60) compared with tumors negative for MAPK (OR = 0.69; 95% CI, 0.41–1.15). No heterogeneity by MAPK expression was observed for parity, oral contraceptive use, age at menarche, menopausal status, or age at menopause (Table 2).

When joint p53/MAPK status was examined, significant heterogeneity was observed for family history of breast or ovarian cancer and parity (Table 3). A family history of breast or ovarian cancer was associated with a significant increased risk for p53-mutant/MAPK-negative tumors (OR = 2.33; 95% CI, 1.44–3.75), a nonsignificant increased risk of p53 wild-type/MAPK-negative tumors (OR = 1.63; 95% CI, 0.84–3.17), and nonsignificant decreased risks for p53-mutant/MAPK-positive tumors (OR = 0.75; 95% CI, 0.40–1.40) and p53 wild-type/MAPK-positive (OR = 0.70; 95% CI = 0.25–2.03; $P_{\text{heterogeneity}} = 0.01$). Compared with nulliparous women, parous women had a strong decreased risk of p53 wild-type/MAPK-negative (OR = 0.25; 95% CI = 0.13–0.50) and p53 wild-type/MAPK-positive (OR = 0.47; 95% CI, 0.17–1.30); no significant associations were noted for the other tumor types ($P_{\text{heterogeneity}} = 0.01$).

Discussion

In this study, we observed few differences in the associations between lifestyle and reproductive factors and ovarian cancer risk by p53 or MAPK expression. However, family history of breast or ovarian cancer was associated with MAPK-negative tumors (particularly those that were also p53 mutant), but not MAPK-positive tumors, while estrogen plus progesterone HT use was suggestively inversely associated with MAPK-negative tumors and had a suggestive positive association with MAPK-positive tumors. In addition, the protective effects of parity were most apparent for p53 wild-type tumors. None of these observed difference in associations was fully explained by histologic subtype and grade.

Ovarian cancer is a heterogeneous disease that has been hypothesized to arise from two distinct developmental pathways.

Table 1. Selected characteristics by p53 and MAPK expression in invasive ovarian tumors in the NHS, NHS II, and NECC

	Controls (n = 1,907)	p53 wild-type (n = 88)	p53 mutant ^a (n = 186)	MAPK ⁻ (n = 142)	MAPK ^{+a} (n = 132)
Mean (SD)					
Age at diagnosis	57.2 (11.2)	58.2 (9.5)	60.9 (9.9)	59.3 (9.7)	60.8 (10.0)
N (%)					
Study					
NHS	866 (45.4)	56 (63.6)	114 (61.3)	89 (62.7)	81 (61.4)
NHS II	184 (9.6)	14 (15.9)	18 (9.7)	16 (11.3)	16 (12.1)
NECC	857 (44.9)	18 (20.5)	54 (29.0)	37 (26.1)	35 (26.5)
Family history of breast or ovarian cancer	306 (16.0)	16 (18.2)	39 (21.0)	39 (27.5)	16 (12.1)
Age at menarche					
<13 years	927 (48.6)	39 (44.3)	86 (46.2)	67 (47.2)	58 (43.9)
13+ years	979 (51.4)	49 (55.7)	100 (53.8)	75 (52.8)	74 (56.1)
Oral contraceptive use					
Never	762 (40.3)	39 (45.3)	97 (52.4)	69 (48.9)	67 (51.5)
<1 year	205 (10.8)	11 (12.8)	24 (13.0)	16 (11.3)	19 (14.6)
1-4 years	421 (22.3)	23 (26.7)	41 (22.2)	39 (27.7)	25 (19.2)
5-9 years	280 (14.8)	10 (11.6)	17 (9.2)	11 (7.8)	16 (12.3)
10+ years	222 (11.7)	3 (3.5)	6 (3.2)	6 (4.3)	3 (2.3)
Tubal ligation	404 (21.2)	10 (11.4)	19 (10.2)	19 (13.4)	10 (7.6)
Parity					
Nulliparous	207 (10.9)	19 (21.8)	17 (9.1)	21 (14.8)	15 (11.5)
1-2 children	812 (42.6)	38 (43.7)	81 (43.5)	67 (47.2)	52 (39.7)
3-4 children	716 (37.6)	20 (23.0)	63 (33.9)	37 (26.1)	46 (35.1)
5+ children	171 (9.0)	10 (11.5)	25 (13.4)	17 (12.0)	18 (13.7)
Menopausal status					
Premenopausal/Unknown	626 (32.8)	24 (27.3)	39 (21.0)	40 (28.2)	23 (17.4)
Postmenopausal	1,281 (67.2)	64 (72.7)	147 (79.0)	102 (71.8)	109 (82.6)
Age at menopause ^b					
<50	413 (36.9)	24 (46.2)	37 (34.3)	30 (40.5)	31 (36.0)
50+	707 (63.1)	28 (53.8)	71 (65.7)	44 (59.5)	55 (64.0)
Ever hormone therapy (HT) use ^b	551 (46.3)	35 (58.3)	71 (57.3)	48 (53.3)	58 (61.7)
Ever estrogen only	237 (19.9)	20 (33.3)	44 (35.5)	31 (34.4)	33 (35.1)
Ever estrogen and progesterone	340 (28.6)	19 (31.7)	35 (28.2)	20 (22.2)	34 (36.2)
Ever other HT	121 (10.2)	7 (11.7)	11 (8.9)	11 (12.2)	7 (7.4)
Histology and grade					
Serous, low grade		4 (4.6)	4 (2.2)	4 (2.8)	4 (3.0)
Serous, high grade		35 (39.8)	133 (71.5)	80 (56.3)	88 (66.7)
Endometrioid, low grade		11 (12.5)	5 (2.7)	13 (9.2)	3 (2.3)
Endometrioid, high grade		15 (17.1)	18 (9.7)	14 (9.9)	19 (14.4)
Mucinous		1 (1.1)	3 (1.6)	3 (2.1)	1 (0.8)
Clear cell		18 (20.5)	6 (3.2)	14 (9.9)	10 (7.6)
Other		4 (4.6)	17 (9.1)	14 (9.9)	7 (5.3)
MAPK positive		34 (38.6)	98 (52.7)	—	—
p53 mutant		—	—	88 (62.0)	98 (74.2)

NOTE: Missing (age at menarche $N = 1$, OC use $N = 20$, parity $N = 2$, age at natural menopause among postmenopausal women $N = 212$, HT use among postmenopausal women $N = 118$).

^aMutant p53 defined as 0% of cells stained positive or >50% stained positive. MAPK was considered positive if >10% of cells stained positive.

^bAmong postmenopausal women.

In this analysis, we used p53 and MAPK immunohistochemistry expression as potential proxy markers for type I and type II ovarian cancers. To our knowledge, no other studies have examined associations with established ovarian cancer risk factors stratifying tumors by these markers. In previous analyses using the NECC study, histologic subtype and grade was used to classify cases as type I ($n = 358$) or type II ($n = 1,108$). Consistent with our results of a strong association for p53 wild-type tumors (hypothesized to be type I), Merritt and colleagues observed that parity was more strongly inversely associated with type I tumors, defined as low-grade serous, low-grade endometrioid/mixed, mucinous, and clear-cell tumors (4). Fortner and colleagues also reported a stronger inverse association with parity among type I cases ($P_{\text{heterogeneity}} = 0.02$) in the European

Prospective Investigation into Cancer and Nutrition (EPIC) cohort (6). In an analysis combining 21 prospective cohorts, Wentzensen and colleagues also observed stronger inverse associations for parity with endometrioid, clear cell, and mucinous carcinomas (subtypes more likely to be type I tumors) compared with serous tumors (5). Overall, there is strong evidence that parity may be most important for this developmental pathway. This is consistent with a theory regarding the "wash-out" effect, wherein a pregnancy, likely due to high progesterone exposure, clears malignant cells of an ovarian/endometrial origin from the ovary that could ultimately develop into type I tumors (21). However, limited evidence from mouse models does directly not support this theory, as models progesterone has been demonstrated to suppress the growth of p53-null ovarian tumors

Table 2. Multivariable^a ORs and 95% CIs for invasive ovarian cancer by p53 expression and MAPK expression

	All (n = 274)	p53 wild-type (n = 88)	p53 mutant ^b (n = 186)	P _{het}	MAPK ⁻ (n = 142)	MAPK ⁺ ^b (n = 132)	P _{het}
Family history of breast or ovarian cancer							
No	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
Yes	1.36 (0.98–1.88)	1.23 (0.70–2.17)	1.41 (0.97–2.07)	0.68	2.06 (1.39–3.06)	0.74 (0.43–1.27)	<0.01
Parity							
Nulliparous	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
Parous	0.60 (0.40–0.90)	0.31 (0.18–0.55)	0.92 (0.54–1.59)	<0.01	0.51 (0.30–0.86)	0.73 (0.41–1.30)	0.35
Nulliparous	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
1–2 children	0.71 (0.46–1.08)	0.40 (0.22–0.73)	1.05 (0.60–1.85)	0.02	0.66 (0.38–1.12)	0.78 (0.42–1.43)	0.67
3–4 children	0.45 (0.29–0.71)	0.20 (0.10–0.39)	0.74 (0.41–1.33)	<0.01	0.33 (0.18–0.60)	0.63 (0.33–1.19)	0.13
5+ children	0.69 (0.40–1.18)	0.37 (0.16–0.87)	1.03 (0.52–2.05)	0.05	0.54 (0.26–1.10)	0.90 (0.42–1.93)	0.31
Oral contraceptive use							
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
Ever	0.80 (0.60–1.07)	0.93 (0.57–1.51)	0.75 (0.54–1.06)	0.47	0.84 (0.57–1.23)	0.77 (0.52–1.14)	0.74
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
1–11 months	0.97 (0.64–1.47)	0.96 (0.47–1.97)	0.97 (0.59–1.59)	0.98	0.86 (0.48–1.54)	1.08 (0.62–1.88)	0.56
1–4 years	1.04 (0.73–1.47)	1.30 (0.73–2.32)	0.94 (0.62–1.42)	0.35	1.26 (0.80–1.97)	0.81 (0.48–1.34)	0.18
5–9 years	0.68 (0.43–1.08)	0.87 (0.41–1.86)	0.61 (0.35–1.07)	0.43	0.56 (0.28–1.10)	0.81 (0.44–1.47)	0.41
10+ years	0.29 (0.14–0.58)	0.30 (0.09–1.02)	0.28 (0.12–0.66)	0.92	0.36 (0.15–0.86)	0.21 (0.06–0.68)	0.46
Tubal ligation							
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
Ever	0.49 (0.32–0.74)	0.55 (0.28–1.09)	0.47 (0.28–0.77)	0.69	0.69 (0.41–1.15)	0.31 (0.16–0.60)	0.06
Age at menarche							
<13 years	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
13+ years	1.15 (0.89–1.50)	1.32 (0.85–2.06)	1.08 (0.80–1.48)	0.45	1.08 (0.77–1.54)	1.25 (0.87–1.79)	0.57
Menopausal status							
Premenopausal/unknown	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
Postmenopausal	1.05 (0.67–1.64)	1.26 (0.60–2.63)	0.96 (0.56–1.67)	0.56	0.78 (0.43–1.41)	1.51 (0.78–2.92)	0.13
Age at menopause							
<50 years	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
50+ years	1.00 (0.70–1.42)	0.77 (0.43–1.37)	1.15 (0.75–1.75)	0.26	0.92 (0.57–1.51)	1.08 (0.67–1.72)	0.65
Hormone therapy (HT) use							
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
Ever	1.50 (1.08–2.08)	1.44 (0.83–2.50)	1.52 (1.03–2.24)	0.87	1.22 (0.78–1.92)	1.82 (1.16–2.84)	0.20
Estrogen-only HT use							
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
Ever	2.03 (1.42–2.90)	1.82 (1.01–3.28)	2.15 (1.42–3.26)	0.63	1.91 (1.18–3.11)	2.17 (1.35–3.49)	0.71
Estrogen + progesterone HT use							
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
Ever	1.08 (0.76–1.53)	1.15 (0.65–2.04)	1.04 (0.69–1.59)	0.79	0.72 (0.43–1.22)	1.52 (0.96–2.38)	0.03
Other HT use							
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	
Ever	0.74 (0.43–1.27)	0.85 (0.37–1.99)	0.68 (0.35–1.34)	0.67	0.95 (0.47–1.89)	0.54 (0.24–1.23)	0.29

^aAdjusted for age (continuous), study (NHS, NHS II, NECC), family history of breast or ovarian cancer (no, yes), oral contraceptive use (months), parity (nulliparous, parous, unknown), parity (continuous), menopausal status and HT use (premenopausal/unknown, postmenopausal no HT use, postmenopausal HT use, postmenopausal HT use unknown).

^bMutant p53 defined as 0% of cells stained positive or >50% stained positive. MAPK was considered positive if >10% of cells stained positive.

(22) and induce necroptosis in p53-deficient cells (23), which would most likely be classified as type II tumors.

Further, we observed a significant positive association for the family history of breast or ovarian cancer with tumors negative for MAPK expression and a nonsignificant inverse association with tumors positive for MAPK expression. This association was particularly strong (OR = 2.30) for tumors that were p53 mutant/MAPK negative, an expression pattern that is most consistent with type II or HGSC tumors. This result is in line with evidence that tumors in BRCA mutation carriers are most likely to be HGSC (24), although it is notable that adjustment for histologic subtype and grade did not appear to explain the strong association between family history and negative MAPK expression.

Estrogen plus progesterone HT had opposite directions of association in relation to MAPK-negative (OR = 0.66; 95% CI, 0.40–1.10) and MAPK-positive tumors (OR = 1.44; 95% CI,

0.95–2.20), though neither reached statistical significance. In contrast, no differential direction was observed for estrogen only HT. The Collaborative Group on Epidemiologic Studies, a collaboration of 52 epidemiologic studies, recently examined the association between HT use and ovarian cancer by histologic subtype. They reported increased risks of both the serous and endometrioid subtypes for estrogen alone and estrogen plus progesterone and decreased risks for clear-cell tumors (25). This is consistent with our results that mediation by histologic subtype and grade did not explain the differential associations for estrogen plus progesterone HT. Further research into the potential mechanisms for how HT use impacts tumor development for both type I and II tumors is warranted.

Limitations of our study include a modest sample size, reducing our power to fully examine mediation by histologic subtype and grade as well as to detect smaller differences in associations. In

Table 3. Multivariable^a ORs and 95% CIs for invasive ovarian cancer by cross-classified p53 and MAPK expression^b

	p53 wild-type/MAPK ⁻ (n = 54)	p53 mutant/MAPK ⁻ (n = 88)	p53 wild-type/MAPK ⁺ (n = 34)	p53 mutant/ MAPK ⁺ (n = 98)	P _{heterogeneity}
Family history of breast or ovarian cancer					
No	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
Yes	1.63 (0.84–3.17)	2.33 (1.44–3.75)	0.70 (0.25–2.03)	0.75 (0.40–1.40)	0.01
Parity					
Nulliparous	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
Parous	0.25 (0.13–0.50)	1.02 (0.45–2.30)	0.47 (0.17–1.30)	0.86 (0.43–1.72)	0.03
Nulliparous	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
1–2 children	0.34 (0.17–0.70)	1.28 (0.56–2.95)	0.56 (0.19–1.61)	0.88 (0.42–1.84)	0.08
3–4 children	0.14 (0.06–0.33)	0.72 (0.30–1.73)	0.36 (0.11–1.12)	0.77 (0.36–1.63)	0.01
5+ children	0.31 (0.11–0.89)	0.98 (0.35–2.71)	0.53 (0.13–2.15)	1.09 (0.45–2.65)	0.25
Oral contraceptive use					
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
Ever	0.95 (0.52–1.76)	0.79 (0.49–1.27)	0.89 (0.42–1.90)	0.73 (0.46–1.15)	0.90
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
1–11 months	0.98 (0.40–2.40)	0.79 (0.37–1.68)	0.93 (0.30–2.91)	1.13 (0.61–2.11)	0.91
1–4 years	1.41 (0.68–2.91)	1.21 (0.70–2.11)	1.16 (0.46–2.92)	0.70 (0.39–1.28)	0.43
5–9 years	0.86 (0.33–2.27)	0.40 (0.15–1.04)	0.91 (0.29–2.92)	0.77 (0.39–1.54)	0.60
10+ years	0.30 (0.07–1.35)	0.40 (0.14–1.16)	0.30 (0.04–2.34)	0.18 (0.04–0.76)	0.85
Tubal ligation					
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
Ever	0.69 (0.30–1.56)	0.70 (0.37–1.32)	0.38 (0.11–1.27)	0.29 (0.13–0.63)	0.29
Age at menarche					
<13 years	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
13+ years	1.09 (0.63–1.90)	1.09 (0.70–1.68)	1.83 (0.89–3.75)	1.10 (0.73–1.66)	0.62
Menopausal status					
Premenopausal/unknown	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
Postmenopausal	1.04 (0.42–2.59)	0.65 (0.31–1.39)	1.87 (0.54–6.53)	1.38 (0.64–2.97)	0.40
Age at menopause					
<50 years	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
50+ years	0.69 (0.33–1.47)	1.13 (0.59–2.15)	0.89 (0.37–2.13)	1.16 (0.67–1.99)	0.69
Hormone therapy (HT) use					
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
Ever	1.38 (0.66–2.88)	1.15 (0.66–1.99)	1.51 (0.68–3.36)	1.95 (1.16–3.30)	0.56
Estrogen only HT use					
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
Ever	1.33 (0.58–3.05)	2.36 (1.32–4.22)	2.55 (1.12–5.84)	2.01 (1.15–3.54)	0.66
Estrogen + progesterone HT use					
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
Ever	1.20 (0.56–2.55)	0.49 (0.23–1.01)	1.09 (0.46–2.55)	1.71 (1.02–2.87)	0.05
Other HT use					
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	
Ever	1.20 (0.43–3.32)	0.81 (0.33–2.01)	0.48 (0.11–2.14)	0.57 (0.22–1.48)	0.67

^aAdjusted for age (continuous), study (NHS, NHS II, NECC), family history of breast or ovarian cancer (no, yes), oral contraceptive use (months), parity (nulliparous, parous), parity (continuous), menopausal status and hormone therapy (HT) use (premenopausal/unknown, postmenopausal no HT use, postmenopausal HT use).

^bMutant p53 defined as 0% of cells stained positive or >50% stained positive. MAPK was considered positive if >10% of cells stained positive.

addition, tumor blocks could not be retrieved for all eligible cases. If the distribution of lifestyle/reproductive factors and tumor characteristics differed between cases by tumor block retrieval status this could result in selection bias. However, cases from which tumor blocks were obtained were generally similar to cases from which tumor blocks were not obtained with respect to the examined factors and tumor characteristics obtained from pathology reports. Independent evaluation in other large epidemiologic studies with tumor tissue is needed to further examine these associations. Further, we only used two tumor stains to estimate tumor developmental pathway. While prior studies have suggested that this may lead to more accurate results than histologic evaluation by a pathologist (26), we may not have fully characterized all the tumors. Further study using an extended staining panel (26) to classify the tumors might be of value.

In conclusion, these findings provide support that leveraging protein expression in tumors can elucidate etiologic pathways of

development in relationship to prediagnosis risk factors. Our findings, which were consistent with those observed by histotype, suggested that parity, family history of breast or ovarian cancer, and estrogen plus progesterone HT use may be associated with tumor subtypes defined by p53 and MAPK expression and these results are not fully explained by histologic subtype and grade. In future studies, other immunohistochemical markers or gene expression profiles that more clearly define types I and II should be considered.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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