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Somatic Mosaic Mutations in *PPM1D* and *TP53* in the Blood of Women With Ovarian Carcinoma

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Abstract

IMPORTANCE—Somatic mosaic mutations in *PPM1D* have been reported in patients with breast cancer, lung cancer, and ovarian cancer (OC), but cause or effect has not been established.

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OBSERVATIONS—To test the hypothesis that somatic mosaic mutations are associated with chemotherapy exposure, we used massively parallel sequencing to quantitate mutations in peripheral blood mononuclear cells (PBMCs) of 686 women with primary OC (n = 412) or relapsed OC (n = 274). The frequency of somatic mosaic *PPM1D* mutations in PBMCs was significantly associated with prior chemotherapy ($P < .001$), and, in patients exposed to chemotherapy, with older age at blood draw (recurrent OC odds ratio [OR], 17.24; 95%CI, 6.80–43.69; and primary OC postchemotherapy OR, 4.82; 95%CI, 1.43–16.18). In contrast, somatic mosaic mutations in *TP53* were not significantly associated with chemotherapy or age. In sequential PBMC samples harvested from 13 patients with OC near diagnosis and after a median of 2 different chemotherapy regimens, somatic mosaic *PPM1D* mutations increased in 11 individuals (84.6%) and *TP53* mutations appeared in 2 (15.4%).

CONCLUSIONS AND RELEVANCE—Chemotherapy exposure and age influence the accumulation of *PPM1D*-mutated PBMC clones. Care should be taken to control for chemotherapy exposure and age at blood draw when testing the association of somatic mosaic mutations in PBMCs with cancer risk.

In 2011, an association between somatic mosaic mutations in the p53-inducible protein phosphatase gene *PPM1D* in peripheral blood mononuclear cells (PBMCs) in patients with breast cancer and ovarian cancer (OC) was reported.[1] All *PPM1D* mutations were truncating mutations in the last exon, were thought to be activating, and were absent from matched cancers. Akbari et al[2] confirmed these findings and suggested that somatic *PPM1D* mutations reveal an OC predisposition that warrants risk-reducing salpingo-oophorectomy. Somatic mosaic *PPM1D* mutations have also been reported in lung cancer.[3] Whether *PPMID* mutations reflect an underlying cancer predisposition or consequence of cancer therapy is unclear. Here we investigated whether *PPM1D* somatic mosaic mutations reflect a previously unrecognized association with prior chemotherapy and whether similar mutations also occur in other genes associated with OC.

Methods

Blood and tumor tissue were collected from patients with OC who provided written consent, including 412 patients with primary OC from 2 institutional tissue repositories and 274 patients enrolled in a Gynecologic Oncology Group clinical trial for recurrent platinum-resistant OC. All protocols were approved by an institutional review board. Cancer-associated genes in PBMC DNA and tumor DNA were sequenced with BROCA (University of Washington)[4–6] to assess the relationship between prior chemotherapy exposure, age, and somatic mosaic mutations. Targeted genes are listed in eTable 1 in the Supplement. Deleterious mutations present at low frequency in *PPM1D*, and other genes were individually examined using the Integrative Genomics Viewer program (Broad Institute) to verify local sequence quality. Variants were included if at least 5 high-quality reads were identified. For 13 women with recurrent OC having a *PMM1D* mutation, DNA from PBMCs obtained close to the time of diagnosis was also sequenced. Validation of low variant calls was performed by resequencing with BROCA at greater depth or, for cases with a greater than 5% variant reads, by Sanger sequencing. Two-tailed *P* values were generated for contingency tables using Fisher exact test and for age comparisons using the exact Wilcoxon

test. Odds ratios were calculated from contingency tables using GraphPad Prism (GraphPad Software).

Results

Targeted sequencing was applied to PBMCs from 686 women with OC. Among 65 genes examined (eTable 1 in the Supplement), somatic mosaic mutations were observed in *PPM1D* in 69 patients (truncating mutations exclusively in exon 6) and in *TP53* in 11 patients (2 frameshift mutations, 8 deleterious missense mutations in the p53DNA binding domain, and 1 missense mutation [p.M44I] predicted to not affect function [http://p53.iarc.fr/TP53GeneVariations.aspx]).

Somatic mosaic *PPM1D* mutations in women with OC were strongly associated with prior chemotherapy exposure ($P < .001$) and, for those patients exposed to chemotherapy, with age at blood draw (Table). The presence of *PPM1D* mutations in patients with relapsed OC was also associated with more previous chemotherapy regimens: *PPM1D* mutations were detected in 21 of 138 women (15.2%) after 1 regimen vs 35 of 130 women (26.9%) after 2 regimens ($P = .02$). The occurrence of multiple different *PPM1D* mutations in the same individual was limited to patients with relapsed OC (14 of 274 women [5.1%] vs 0 of 412; $P < .001$).

Somatic mosaic mutations were not confirmed in any other gene on the BROCA panel except *TP53*. Somatic mosaic *TP53* mutations were present in 4 of 326 women (1.2%) with OC not exposed to previous chemotherapy and 7 of 274 women (2.6%) with recurrent, platinum-resistant OC, and were not significantly associated with chemotherapy exposure ($P = .24$). As chemotherapy was not associated with *TP53* mutations, we evaluated the association with age in the entire cohort of 686 patients with OC. The mean age—67.1 years in the 11 patients with *TP53* mutations and 61.5 years in those without mutations— was not significantly different (Monte Carlo-based Wilcoxon test, $P = .07$). Of the 11 blood samples with somatic mosaic *TP53* mutations, 5 also had *PPM1D* mutations.

Paired neoplastic tissue was available for 4 women with somatic *TP53* mutations and 13 with *PPM1D* mutations. In no case was the PBMC mutation identified in tumor DNA.

To further assess whether treatment influenced the presence or frequency of these somatic mosaic mutations, we sequenced DNA at increased depth from pairs of PBMC samples obtained from 13 patients with somatic mosaic mutations near diagnosis and again at relapse (eTable 2 in the Supplement). Overall, 21 *PPM1D* mutations and 2 *TP53* mutations were either undetectable at diagnosis or present at a lower fraction of reads than at recurrence. In 3 patients, *PPM1D* mutations occurred in a high fraction of reads (30%–37%) at diagnosis and did not increase with chemotherapy exposure.

Key Points

Question

Does chemotherapy exposure influence the presence of somatic mosaic *PPM1D* mutations in the blood of women with ovarian carcinoma?

Findings

In 686 women with ovarian carcinoma, the frequency of somatic mosaic *PPM1D* mutations in peripheral blood mononuclear cells (PBMCs) was significantly associated with prior chemotherapy, and, in patients exposed to chemotherapy, with older age at blood draw.

Meaning

Chemotherapy exposure and age influence the accumulation of *PPM1D*-mutated PBMC clones, and these factors should be controlled for when testing the association of somatic mosaic mutations in PBMCs with cancer risk.

Discussion

Our results demonstrate that somatic *PPM1D* mutations in PBMCs are associated with prior chemotherapy and older age. Somatic *TP53* mutations were also identified in 11 patients (1.6%) but were not significantly associated with chemotherapy or age. Damaging somatic mosaic mutations in other genes on the BROCA panel were not identified. These results shed new light on recent reports linking somatic mosaic *PPM1D* mutations with solid tumor risk.

Previous studies have reported that the presence of *PPM1D* mutations in PBMCs was associated with breast cancer and OC but cause and effect were not established.[1] However, some investigators suggested that identification of such mutations might warrant risk-reducing salpingo-oophorectomy.[2] Somatic mosaic *PPM1D* mutations have also been reported in lung cancer.[3] Although it has been assumed that the presence of these mutations might reflect an underlying repair defect that contributes to cancer predisposition, our results suggest that this association between *PPM1D* mutations and solid tumors might have been confounded by prior chemotherapy. In particular, we observe a stepwise increase in the incidence of *PPM1D* somatic mosaic mutations between patients with OC without prior chemotherapy, patients with newly diagnosed OC but prior chemotherapy exposure, and patients previously treated extensively for OC (Table). Moreover, 11 of 13 pairs of sequential samples show new appearance or increased frequency of *PPM1D* mutations at 21 of 24 individual mutation sites during the course of treatment (eTable 2 in the Supplement). These observations suggest that *PPM1D* somatic mosaic mutations reflect prior chemotherapy exposure. Consistent with this suggestion, all 26 patients with lung cancer and OC with a *PPM1D* mutation previously identified by Akbari et al[2] and Zajkowicz et al[3] also had received chemotherapy before *PPM1D* sequencing. It is possible that there may also be a rare, second group of younger, chemotherapy-naive patients with a more dominant *PPM1D* clone (eg, patients 11–13 in eTable 2 in the Supplement), but the clinical significance of that finding requires additional investigation.

We also identified deleterious *TP53* mutations in PBMCs from patients with OC. While this work was in progress, Wong et al[7] detected *TP53* mutations at very low allele fractions (0.003%–0.7%) in 9 of 19 elderly individuals without cancer, demonstrated in mouse bone marrow chimeras that $TP53^{+/-}$ cells are preferentially expanded after chemotherapy, and

concluded that *TP53* mutations may confer a subtle competitive advantage to nonmalignant clones within the normal marrow, particularly during DNA damaging chemotherapy. In patients with OC, we did not find an association of *TP53* mutations with age or chemotherapy exposure, but the number of PBMC *TP53* mutations was small (n = 11). Notably, in 2 cases, *TP53* mutations emerged after prolonged chemotherapy (eTable 2 in the Supplement), suggesting that there may be an effect of extended chemotherapy on somatic *TP53* mutations, although that effect was not detected in the larger cohort. At the time of PBMC harvest, none of the OC patients with a PBMC *TP53* mutation had evidence of a therapy-related hematologic malignancy.

Conclusions

Given the relationship between prior chemotherapy, age, and somatic mosaic mutations in *PPM1D*, long-term prospective studies appear to be required to determine whether *PPM1D* or *TP53* somatic mosaic mutations by themselves actually confer an increased risk of primary or secondary neoplasms.

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Table

PPM1D Mutations in PBMCs From Women With Primary and Recurrent Ovarian Carcinoma

Ovarian Carcinoma Subgroup	No.	No. With *PPM1D* Mutation (Fraction)	OR (95% CI)	Mean Age, y		*P* Value
				Without *PPM1D* Mutation	With *PPM1D* Mutation	
Primary, no CT	326	5 (.015)[a]	NA	61.8	69.0	.30
Primary, CT exposed	86	6 (.070)[a]	4.82 (1.43–16.18)	55.4	68.2	.02
Recurrent, platinum-resistant	274	58 (.212)[a]	17.24 (6.80–43.69)	62.1	65.7	.01

Abbreviations: CT, chemotherapy; NA, not applicable; OR, odds ratio; PBMCs, peripheral blood mononuclear cells.

[a]Fisher exact test of an equal frequency of *PPM1D* mutations across the 3 chemotherapy groups, $P < .001$; no CT vs CT exposed ($P = .013$); and CT exposed vs recurrent platinum-resistant ovarian carcinoma ($P < .002$).