The *KRAS*-Variant Genetic Test As a Marker of Increased Risk of Ovarian Cancer

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Ovarian cancer (OC) is the fifth leading cause of cancer death in women. High mortality rates are due to vague symptoms of early disease, leading to diagnosis at a late stage. Approximately 10% of OCs are considered hereditary. The KRAS*-variant is an inherited, germline variant that has been demonstrated to serve as a genetic marker of increased risk of OC. The* KRAS*-variant is easily tested in a blood or saliva sample, and has been shown to be at a high prevalence in OC patients. This test could serve as an answer to patients with a family history of ovarian and breast cancer without a previously identifiable genetic mutation.*
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Ovarian cancer (OC) is the fifth leading cause of cancer death in women. In 2009 there were 21,550 new cases and 14,600 deaths.[1] The high mortality rate is due to vague symptoms of early disease, leading to diagnosis at a late stage. Approximately 10% of OCs are considered hereditary. *BRCA1*, *BRCA2*, and hereditary nonpolyposis colorectal cancer (HNPCC) gene mutations have been the only genetic abnormalities known to account for familial OC, but they are extremely rare. Unfortunately, additional gene alterations explaining a significant proportion of the remaining familial risk have not been previously identified.

MicroRNAs (miRNAs) are a class of 22 nucleotide, noncoding RNAs that are evolutionarily conserved and function by negatively regulating gene expression

at the posttranscriptional level. Recent studies have shown that miRNAs are aberrantly expressed in virtually all cancer types.[2] Single nucleotide polymorphisms (SNPs), disrupting miRNAs, or miRNA binding sites have been demonstrated to be individually powerful biomarkers of cancer risk in humans.

The *KRAS*-variant is an inherited, germline variant that has been demonstrated to serve as a genetic marker of increased risk of OC. The *KRAS*-variant is easily tested in a blood or saliva sample; and has been shown to be at a high prevalence in OC patients, being present in over 25% of patients with epithelial OC, compared with 6% to 10% of the general population.[3] The *KRAS*-variant is strongly associated with hereditary breast and ovarian cancer (HBOC) patients, where previous genetic testing did not provide a genetic diagnosis of cancer risk, which was present in 61% of these uninformative families. (Patients diagnosed with what appears to be familial OC syndrome, yet who test negative for these known genetic mutations, are referred to as *uninformatives*.) These findings indicate that the *KRAS*-variant may represent a new genetic marker of OC risk for patients from HBOC families.

MiRNAs

MiRNAs are a class of nonprotein coding, single-strand RNAs approximately 22 nucleotides in length that negatively regulate multiple gene targets. Since the first miRNA was discovered in the roundworm *Caenorhabditis elegans* over 15 years ago,[4] more than 500 conserved miRNAs have been identified in the human genome.[5] It has been estimated that the total number of human miRNAs, including nonconserved miRNAs, is over 700.[6] MiRNAs inhibit gene expression either through the RNA interference pathway, which leads to mRNA degradation, or by binding to the 3′ untranslated region (UTR) of mRNA and blocking protein production during translation.[2,7] MiRNAs are global RNA regulators emerging as important master controllers of cell survival pathways with significance in human cancer.[8] Frequent misregulation of miRNAs in numerous cancers supports the hypothesis that mutation of these miRNAs may be initiating events in cancer, and that specific miRNAs misregulated in each cancer type may act as biomarkers as well as potential targets for future novel therapies.[9] As miRNAs regulate hundreds of messenger RNAs, mutations in the miRNA itself or in its binding site could be associated with malignant transformation or disease progression.

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Oncomirs, which are miRNAs associated with cancer, may function as oncogenes or tumor-suppressor genes. Tumor-suppressor genes include *let-7* in lung cancer, *mir-125b* in breast cancer, and *miR-15a* in B-cell chronic lymphocytic leukemia.[10] Oncogenes include *miR-155* in breast cancer,[11] *miR-21* in glioblastoma,[12] and *miR-155* in Burkitt and Hodgkin lymphoma.[13] In addition, miRNAs have been found to predict prognosis and response to therapy.[10,14]

There is evidence that SNPs in miRNA binding sites can be associated with disease. For example, a point mutation identified in several Tourette syndrome patients in the 3′UTR of *SLITRK1* disrupts the binding of *miR-189*.[15] Supporting evidence was demonstrated by He and colleagues[7] and Landi and associates who confirmed that allele frequencies in the 3′UTR of many genes vary between cancerous and normal samples.

The miRNA *let-7* family, which functions as a tumor suppressor, negatively regulates the *RAS* pathway and *HMGA2*.[16] Deregulation of the *let-7* family occurs in several cancers, including lung, colon, breast, ovarian, pancreatic, and prostate.[17]

KRAS-Variant

We previously identified a germline single nucleotide polymorphism (rs61764370 T > G) in the *let-7* complementary site 6 in the *KRAS* 3′UTR region. To assess the impact of the *KRAS*-variant on *KRAS* expression, A549 cells, a lung cancer cell line, were transfected with a luciferase reporter containing the *KRAS*-variant in the *KRAS* 3′UTR and with a luciferase reporter containing the wild-type *KRAS* 3′UTR. There was increased *KRAS* expression in the cells transfected with the *KRAS* variant than in cells transfected with the wild-type *KRAS* 3′UTR. Therefore, the *KRAS*-variant disrupts the binding of *let-7* to *KRAS*, leading to increased *KRAS* expression, and is associated with lower *let-7* levels in tumors.

The *let-7* family of miRNAs acts as a tumor suppressor. It has been demonstrated in lung cancer that *let-7* is reduced in cancer tissue, and *RAS* is elevated.[18] The *KRAS*-variant is found in 5.3% of the world's population, and in 12% of white populations of European descent, based on the genotyping of more than 2500 samples representing 46 geographic populations.[18] Moreover, in patients with a moderate smoking

history, defined as < 41 pack years, the *KRAS*-variant was associated with a 1.4- to 2.3-fold increased risk (odds ratio [OR] 1.4; 95% confidence interval [CI], 1.1-1.7; $P = .01$; OR 2.3; 95% CI, 1.1-4.6; $P = .02$) of non-small cell lung cancer.[19]

The *KRAS*-variant has also been shown to predict outcome and response to therapy in other cancers. In patients with oral squamous cell cancers, the *KRAS*-variant was a statistically significant predictor for reduced overall survival (OS) (hazard ratio 2.7; 95% CI, 1.4-5.3).[20] Among patients with irinotecan-refractory metastatic colorectal cancer treated with anti-epidermal growth factor inhibitor therapy, OS and progression-free survival were both significantly decreased in patients with the *KRAS*-variant.[21]

The *KRAS*-Variant and OC

OC is the second most common gynecologic malignancy, but the most common cause of death from gynecologic malignancies. [1] Over 90% of OCs are epithelial OCs, which arise from the epithelial layer of the ovary. Epithelial OC is the fifth leading cause of cancer death in women in the United States, with over 15,000 women dying annually of this disease. Approximately 22,000 women are diagnosed with OC each year, representing a lifetime risk of developing OC of 1 in 71. Early symptoms are vague, and approximately 75% of patients have stage III or stage IV disease at the time of diagnosis. For those patients with ovarian adenocarcinoma, the 5-year survival rate is 33.5% (stage III) and 17.9% (stage IV).[22] Randomized trials conducted by the Gynecologic Oncology Group[23] have demonstrated that prognosis worsens in epithelial OC with older age, higher grade, higher stage, and malignant cytology. Serial monitoring of cancer antigen 125 levels is currently used in patient follow-up, but is not without controversy given its low sensitivity in early disease.[24] As such, further identification and study of molecular markers is key to providing a more thorough assessment of cancer risk, patient prognosis, and response to therapy.

There is an inherited component of risk for developing OC, as first-degree relatives of OC patients are at a higher risk of developing disease than the general population.[25] These familial cases are thought to make up approximately 10% of all OC, with the rest of cases considered as sporadic. There are 2 previously defined hereditary syndromes that include predisposition to OC: HBOC, which is associated with mutations in the tumor suppressor genes *BRCA1* and *BRCA2*, and HNPCC, associated with mutations in *MSH2*, *MLH1*, and *MSH6*, which together account for no more than 10% of all OC cases.

Patients diagnosed with what appears to be familial OC syndrome, yet who test negative for these known genetic mutations, are referred to as uninformatives.

As the *KRAS*-variant acts as a genetic marker of cancer risk, which is likely due to the importance of *let-7* and *KRAS* in human cancer. Thus the prevalence of the *KRAS*-variant in patients with additional solid tumor types was tested, including breast, colon, pancreatic, prostate, uterine, and ovarian. The *KRAS*-variant was shown to be present in over 25% of patients with epithelial OC compared with < 18% in control populations or other cancerous populations with solid tumors. Case control analyses also demonstrated an increased OC risk in *KRAS*-variant patients (OR 2.46; 95% CI, 1.14-5.29; $P = .020$).[3]

To further assess the impact of the *KRAS*-variant on OC risk, OC patients with a family history consistent with HBOC who were tested previously for *BRCA1* and *BRCA2* were evaluated. The *KRAS*-variant was present in 61% of the uninformative patients with OC, a frequency that was significantly higher than in OC patients without a family history ($P < .001$).[3] These findings indicate that the *KRAS*-variant may represent a new genetic marker of familial OC risk, accounting for a large number of HBOC families previously considered uninformative.

The family profiles of *KRAS*-variant patients were also different than *BRCA1* or *BRCA2* patients. Specifically, the patients in *KRAS*-variant HBOC families were more likely to be older at the time of diagnosis, non-Jewish, and to have a family history of lung cancer.

Conclusions

The *KRAS*-variant has been shown to be a genetic marker of an increased risk of developing OC. This variant is significantly enriched in families previously considered uninformative with HBOC, suggesting that the *KRAS*-variant may be a new genetic marker of risk for these families without prior identified genetic risk. The *KRAS*-variant test could serve as an answer to patients with HBOC without a previously identifiable genetic mutation.

A patient's *KRAS*-variant status can be tested using a commercially available blood test (*PreOvar*™, MiraDx, New Haven, CT). The *KRAS*-variant is present in 6% to 10% of the general population and more than 25% of nonselected women with epithelial OC. Additionally, the *KRAS*-variant was identified in over 60% of HBOC patients who were previously classified as uninformative, or negative for other known genetic markers of OC risk. *PreOvar*, by testing for the *KRAS*-variant, will help identify OC

patients who have this variant and whose first- and second-degree relatives may be appropriate for testing. *PreOvar* may also help assess the relative risk of developing OC for women who have a family history of OC without an alive proband. The role of the *KRAS*-variant as a biomarker of outcome for patients diagnosed with OC is currently under investigation. Reports by others have demonstrated that the *KRAS*-variant predicts poor outcome as well as resistance to certain therapies.

The MiraDx *PreOvar* test requires a saliva or blood sample and results are confirmed within 5 days. The report will identify the existence of the *KRAS*-variant as a positive or negative result. Patients with the *KRAS*-variant should be managed in a manner similar to those with a *BRCA* mutation. ■

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Main Points

- The *KRAS*-variant is an inherited, germline variant that has been demonstrated to serve as a genetic marker of increased risk of ovarian cancer (OC). The *KRAS*-variant is easily tested in a blood or saliva sample, and has been shown to be at a high prevalence in OC patients.
- Epithelial OC is the fifth leading cause of cancer death in women in the United States, with over 15,000 women dying annually of this disease. Approximately 22,000 women are diagnosed with OC each year, representing a lifetime risk of developing OC of 1 in 71.
- Randomized trials conducted by the Gynecologic Oncology Group have demonstrated that prognosis worsens in epithelial OC with older age, higher grade, higher stage, and malignant cytology. Further identification and study of molecular markers is key to providing a more thorough assessment of cancer risk, patient prognosis, and response to therapy.
- To assess the impact of the *KRAS*-variant on OC risk, patients with a family history consistent with hereditary breast and ovarian cancer (HBOC) who were tested previously for *BRCA1* and *BRCA2* were evaluated. The *KRAS*-variant was present in 61% of the uninformative patients with OC, a frequency that was significantly higher than in OC patients without a family history ($P < .001$).
- The *KRAS*-variant has been shown to be a genetic marker of an increased risk of developing OC. It is significantly enriched in families previously considered uninformative with HBOC, suggesting that the *KRAS*-variant may be a new genetic marker of risk for these families without prior identified genetic risk. The *KRAS*-variant test could serve as an answer to patients with a family history of HBOC without a previously identifiable genetic mutation.