Research Article

Gene Variants in Angiogenesis and Lymphangiogenesis and Cutaneous Melanoma Progression

Jong Y. Park1, Ernest K. Amankwah1, Gabriella M. Anic1, Hui-Yi Lin2, Brooke Walls3, Hyun Park4, Kevin Krebs5, Melissa Madden5, Kristen Maddox6, Suroosh Marzbani2, Shenying Fang7, Wei Chen8, Jeffrey E. Lee9, Qingyi Wei8, Christopher I. Amos8, Jane L. Messina5,6, Vernon K. Sondak3, Thomas A. Sellers1, and Kathleen M. Egan1

Abstract

Background: Angiogenesis and lymphangiogenesis are important in the progression of melanoma. We investigated associations between genetic variants in these pathways with sentinel lymph node (SLN) metastasis and mortality in 2 independent series of patients with melanoma.

Methods: Participants at Moffitt Cancer Center were 552 patients, all Caucasian, with primary cutaneous melanoma referred for SLN biopsy. A total of 177 patients had SLN metastasis, among whom 60 died from melanoma. Associations between 238 single-nucleotide polymorphisms (SNP) in 26 genes and SLN metastasis were estimated as ORs and 95% confidence intervals (CI) using logistic regression. Competing risk regression was used to estimate HRs and 95% CI for each SNP and melanoma-specific mortality. We attempted to replicate significant findings using data from a genome-wide association study comprising 1,115 patients with melanoma who were referred for SLN biopsy from MD Anderson Cancer Center (MDACC), among whom 189 patients had SLN metastasis and 92 patients died from melanoma.

Results: In the Moffitt dataset, we observed significant associations in 18 SNPs with SLN metastasis and 17 SNPs with mortality. Multiple SNPs in COL18A1, EGF receptor (EGFR), FLT1, interleukin (IL)-10, platelet-derived growth factor D (PDGFD), PIK3CA, and toll-like receptor (TLR)-3 were associated with the risk of SLN metastasis and/or patient mortality. The MDACC data set replicated an association between mortality and rs2220377 in PDGFD. Furthermore, in a meta-analysis, 3 additional SNPs were significantly associated with SLN metastasis (EGFR rs723526 and TLR3 rs3775292) and melanoma-specific death (TLR3 rs7668666).

Conclusions: These findings suggest that genetic variation in angiogenesis and lymphangiogenesis contributes to regional nodal metastasis and progression of melanoma.

Impact: Additional research attempting to replicate these results is warranted. Cancer Epidemiol Biomarkers Prev; 22(5); 827–34. ©2013 AACR.

Introduction

Melanoma incidence is rising in the United States and throughout the world. In the United States, an estimated 76,250 people will be diagnosed with cutaneous melanoma in 2012 (1) and by 2015, the estimated lifetime risk of developing this disease will be 1 in 50 (2, 3). These estimates may underestimate the true incidence of melanoma due to the large number of patients treated in the outpatient setting. Physicians diagnosing new cases of melanoma in the outpatient setting are required to report to the State’s cancer registry. However, half of the dermatologists are not aware of this obligation and therefore a large number of melanomas are not reported. Thus, United States melanoma statistics markedly underestimate the true incidence of the disease (4).

Melanoma is most commonly diagnosed at a clinically localized stage when it is highly curable by surgical removal. However, not all patients present with localized disease. Moreover, determining the risk of metastasis in patients diagnosed with localized melanoma continues to pose a challenge to both clinicians and pathologists (5). A combination of clinical and pathologic features is used for predicting outcomes in patients with localized melanoma. The most important prognostic features include tumor thickness, ulceration, Clark level of invasion, and mitotic rate (6–9). However, these prognostic factors are limited in their ability to reliably distinguish patients that will
manifest regional lymph node metastasis or develop widely disseminated disease and hence are at high risk of dying from melanoma. Currently, the most important predictor of distant metastasis and survival in patients with melanoma is sentinel lymph node (SLN) metastasis (10–12). However, SLN biopsy requires examination of multiple microscopic SLN sections (5) and carries risk including wound complications, mild to moderate degrees of lymphedema, thrombophlebitis, and loss of range of motion (9, 13). There is therefore an urgent need to identify new predictive and prognostic biomarkers (5, 14) to improve prediction models and to target therapies to individual patients with melanoma.

Angiogenesis, the development of new blood vessels, and lymphangiogenesis, the development of new lymphatic vessels or the activation of preexisting vessels, promote tumor growth and metastasis (15, 16). These 2 related processes are governed by many of the same genes (17–19) and the expression of these genes or their protein products has been correlated with tumor stage, progression, metastasis, and survival in melanoma (5, 14, 20–27). It is therefore plausible that genetic variations in components of these 2 pathways may contribute to interindividual differences in melanoma progression and survival.

Although strong biologic rationale exists for the involvement of angiogenesis and lymphangiogenesis in melanoma, few studies have examined the association between genetic variants in these pathways and melanoma progression and survival. Suggestive findings have been reported in studies examining the association of matrix metalloproteinase (MMP)-9 single-nucleotide polymorphisms (SNP) with intransit metastasis (28) and tumor regression (29), MMP-1 SNPs with progression and prognosis (30), VEGF SNP with tumor growth (31), interleukin (IL)-1β SNP with thinner invasive melanoma (32), EGF functional SNP with tumor regression (23), and IL-10 SNP with advanced disease (33). However, no study to date has examined associations of SNPs in these pathways with metastasis to SLNs.

In this study, we examined the association between 238 SNPs in 26 angiogenesis and lymphangiogenesis genes and the risk of SLN metastasis and mortality using data from 552 patients referred to the Moffitt Cancer Center (Tampa, FL) for SLN biopsy for purposes of melanoma staging. We sought to replicate the statistically significant results in an independent population of 1,115 patients with melanoma who had been genotyped as part of a genome-wide association study (GWAS) at MD Anderson Cancer Center (MDACC; Houston, TX).

Materials and Methods

Study patients and data collection

A total of 572 Caucasian patients underwent wide excision surgery for pathologically confirmed cutaneous melanoma followed by SLN biopsy between 1994 and 2010 at the Moffitt Cancer Center and 552 (96.5%) had sufficient material available for the analysis. A total of 177 patients had pathologically documented metastasis to the SLN (SLN-positive) and 375 patients were negative for SLN metastasis (SLN-negative). Demographic and clinical information including age at diagnosis, gender, tumor site, histology, Breslow thickness, ulceration, and Clark level of invasion were obtained from medical records. Date of death, cause of death, and vital status information were obtained from the Moffitt Tumor Registry and through a search of the National Death Index through December 2009. The study was approved by the Institutional Review Board of the University of South Florida (Tampa, FL). We attempted to replicate our statistically significant SNP results using data from a GWAS at MDACC (34). Among 1,952 Caucasian patients with pathologically confirmed melanoma included in the MDACC GWAS, SLN biopsy was conducted on 1,115 cases and SLN metastasis identified in 189. Demographic and clinical information were obtained from a prospectively maintained database. Date of death and vital status information were obtained from the medical record, the MDACC Tumor Registry, and a search of the National Death Index through October 2011.

Genotyping for Moffitt samples

For the initial study, genomic DNA was extracted from formalin-fixed paraffin-embedded tissue blocks obtained from the Tissue Core Facility at the Moffitt Cancer Center. The study pathologist (J.L. Messina, Moffitt Cancer Center) reviewed 5 μm sections of each tissue block and selected blocks containing only nontumor tissue. DNA extractions were carried out using the DNeasy Tissue Kit (Qiagen) according to the manufacturer’s protocol.

Twenty-six genes encoding proteins involved in angiogenesis and lymphangiogenesis were identified from the published literature (35, 36) and public pathway database (Cancer Genome Anatomy Project, Kyoto Encyclopedia of Gene and Genomes and Gene Ontology) searches. Genes differentially expressed between melanoma tumor and normal tissue or involved in the progression of melanoma or other tumor types were selected. TagSNPs (r² > 0.8) and putative functional SNPs with minor allele frequencies (MAF) of 0.05 or more were then selected from the unrelated Caucasian sample within the HapMap Consortium release 27 (37). A total of 261 SNPs (Supplementary Table S1) in both pathways were genotyped using the Illumina GoldenGate assay (Illumina) at the Center for Genome Technology at the Hussman Institute for Human Genomics, University of Miami (Miami, FL). Of the 261 SNPs, 23 failed, resulting in 238 SNPs that were genotyped successfully. Genotyping was attempted on 572 patient DNA samples. Among these samples, we excluded 20 with call rates less than 80% (3.5%; 1 SLN-positive case, 18 SLN-negative cases, and 1 case with no pathologic data), resulting in 552 samples that were successfully genotyped (177 SLN-positive cases and 375 SLN-negative cases). The concordance for 3 duplicate samples was more than 99%.
Statistical analysis for Moffitt samples

Demographic and clinical characteristics of the participants were compared between SLN-positive and -negative cases. Age at diagnosis was summarized using mean and SD, and the Student t test was used to compare mean age at diagnosis between SLN-positive and -negative cases. Categorical variables were summarized using numbers and percentages, and \( \chi^2 \) test/Fisher exact tests were used to test for differences between groups.

The likelihood ratio test in logistic regression was used to estimate associations between SNPs in angiogenesis genes and SLN metastasis. For evaluating SNPs associated with melanoma death, the competing risk regression approach was applied. Survival time was determined as the number of months between date of SLN biopsy and date of death due to melanoma (primary event), death due to other causes, or last contact for censored observations. All models were adjusted for age at diagnosis and Breslow depth. Statistical tests were 2-sided with an \( \alpha \) level less than 0.05 considered statistically significant. For each SNP, the minimum \( P \) value over 3 genetic models (additive, dominant, and recessive models) was used to represent

<table>
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<tr>
<th>Variable</th>
<th>Moffitt</th>
<th>MD Anderson</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>SLN(^a)-positive</td>
<td>SLN(^a)-negative</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>55.9 ± 17.3</td>
<td>58.6 ± 15.2</td>
</tr>
<tr>
<td>Median (range)</td>
<td>57 (15–89)</td>
<td>60 (17–88)</td>
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<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>108 (61.0)</td>
<td>222 (59.2)</td>
</tr>
<tr>
<td>Female</td>
<td>69 (39.0)</td>
<td>153 (40.8)</td>
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<tr>
<td>Histology</td>
<td></td>
<td></td>
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<tr>
<td>Acral lentiginous</td>
<td>8 (4.5)</td>
<td>9 (2.4)</td>
</tr>
<tr>
<td>Desmoplastic</td>
<td>7 (4.0)</td>
<td>16 (4.3)</td>
</tr>
<tr>
<td>Lentigo maligna</td>
<td>1 (0.6)</td>
<td>7 (1.9)</td>
</tr>
<tr>
<td>Nodular</td>
<td>52 (29.4)</td>
<td>109 (29.1)</td>
</tr>
<tr>
<td>Superficial spreading</td>
<td>88 (49.7)</td>
<td>212 (56.5)</td>
</tr>
<tr>
<td>Unknown</td>
<td>21 (11.9)</td>
<td>22 (5.9)</td>
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<td>Ulceration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>53 (34.9)</td>
<td>74 (21.0)</td>
</tr>
<tr>
<td>No</td>
<td>99 (65.1)</td>
<td>278 (78.9)</td>
</tr>
<tr>
<td>Clark level</td>
<td></td>
<td></td>
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<tr>
<td>II</td>
<td>1 (0.6)</td>
<td>6 (1.6)</td>
</tr>
<tr>
<td>III</td>
<td>20 (11.3)</td>
<td>51 (13.6)</td>
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<td>IV</td>
<td>134 (75.7)</td>
<td>300 (80.0)</td>
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<tr>
<td>V</td>
<td>16 (9.0)</td>
<td>16 (4.3)</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (3.4)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>Breslow thickness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 mm</td>
<td>22 (12.6)</td>
<td>80 (21.4)</td>
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<tr>
<td>1–&lt;3 mm</td>
<td>116 (66.3)</td>
<td>240 (64.2)</td>
</tr>
<tr>
<td>≥3 mm</td>
<td>37 (21.1)</td>
<td>54 (14.4)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (1.1)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extremities</td>
<td>82 (46.3)</td>
<td>170 (45.3)</td>
</tr>
<tr>
<td>Trunk</td>
<td>67 (37.9)</td>
<td>133 (35.5)</td>
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<tr>
<td>Head and neck</td>
<td>28 (15.8)</td>
<td>72 (19.2)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<tr>
<td>Melanoma death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>41 (23.2)</td>
<td>19 (5.0)</td>
</tr>
<tr>
<td>No</td>
<td>136 (76.8)</td>
<td>356 (95.0)</td>
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</table>

\(^a\)Sentinel lymph node.

\(^b\)test for a numeric variable and \( \chi^2 \) or Fisher exact test for a categorical variable.
the best-fitting model. For multiple comparison adjustment, the false discovery rate (FDR) q value (38) for each SNP was evaluated. Competing risks regression was conducted using the cmprsk R package, and other statistical analyses were implemented with SAS 9.2 (SAS Institute).

**Genotyping and data quality control for MDACC samples**

Samples were collected from the University of Texas MDACC. DNA samples for the first-stage GWAS were genotyped using the Illumina HumanOmni1-Quad_v1-0_B array and were called using the BeadStudio algorithm, at the John Hopkins University Center for Inherited Disease Research (CIDR; Baltimore, MD). We were able to satisfactorily analyze 1,012,904 of 1,016,423 SNPs attempted (99.6%) with a mean sample call rate of 99.86%. SNPs with MAF ≤ 0.01 and call rate < 95% were excluded. After applying the above criteria, 818,237 genotyped autosomal SNPs were available for the final association analysis. Samples with more than 10% missing rate across all SNPs (n = 41), with identity problem (n = 11), unexpectedly (n = 67) or unexpectingly (n = 5) duplicated or related (n = 15) identified using identity-by-descent coefficient in PLINK, or identified as outliers by principal component analysis (n = 39) were removed. Imputation of ungenotyped SNPs in the whole genome was conducted through MACH using HapMap reference data with a denser set of markers. We were able to fill in the untyped markers in the study subjects by means of maximum likelihood estimation. After imputation, we had 2.65 million SNPs available for this analysis. The average posterior probability for the most likely genotype was 0.99.

**Statistical analysis for MDACC set**

All statistical analyses were conducted by ProbABEL software (39). All genetic effects (additive, dominant, and recessive) for the variant allele of each SNP on SNL metastasis were examined via likelihood ratio test under the null hypothesis of χ² distribution with one degree of freedom. Logistic regression models were built to estimate all 3 genetic models of reference allele on risk of SNL metastasis. Cox regression model was used to estimate genetic effects of genetic polymorphisms on overall survival. All models in Moffitt and MD Anderson data were adjusted for age at diagnosis and Breslow depth.

**Combined meta-analysis**

A meta-analysis was conducted in Stata (version 8.2) to combine results across the 2 studies when ORs were in the same direction and the P value for each study was less than 0.1. The Q test of heterogeneity was estimated to quantify the proportion of total variation due to heterogeneity across studies. No significant heterogeneity was observed between the studies (all P > 0.33). Combined ORs, 95% confidence intervals (CI), and P values were

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP ID</th>
<th>Model</th>
<th>P</th>
<th>a/A</th>
<th>MAFb</th>
<th>OR</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>PIK3CA</td>
<td>rs1607237</td>
<td>Add</td>
<td>0.015</td>
<td>C/G</td>
<td>0.36</td>
<td>1.39 (1.07–1.82)</td>
<td></td>
</tr>
<tr>
<td>VEGFA</td>
<td>rs8036068</td>
<td>Add</td>
<td>0.043</td>
<td>T/C</td>
<td>0.36</td>
<td>0.75 (0.56–0.99)</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP ID</th>
<th>Model</th>
<th>P</th>
<th>a/A</th>
<th>MAFb</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL18A1</td>
<td>rs2838907</td>
<td>Add</td>
<td>0.015</td>
<td>C/G</td>
<td>0.36</td>
<td>1.39 (1.07–1.82)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Odds ratios (ORs) for genetic variants in angiogenesis genes associated with sentinel node metastasis

*a* minor allele; A, major allele.

bMAF, minor allele frequency.

OR (95% CI), OR and 95% CI adjusted for age at diagnosis and Breslow thickness.

Imputed SNPs in MDACC.
generated using inverse-variance weighting to calculate a fixed-effect model.

Results

The mean ages at diagnosis of SLN-positive subjects was nonsignificantly lower than those of SLN-negative subjects in both data set (P = 0.08, Table 1). The distributions of males and females and tumor site did not differ between SLN-positive and negative subjects (Table 1). As expected, ulceration, higher level of invasion (Clark level V) and thicker (Breslow ≥3 mm) tumors, and melanoma-related deaths were more common in SLN-positive than SLN-negative subjects.

The MAFs of successfully genotyped SNPs ranged from 0.14 to 0.48. In the Moffitt data, significant associations (raw P < 0.05, maximum FDR q = 0.085) were observed for 18 SNPs in 11 genes for SLN metastasis: 4 SNPs in PIK3CA, 3 SNPs in COL18A1, 2 SNPs in platelet-derived growth factor D (PDGFD) and FLT1 and 1 SNP in CXCL12, EGF receptor (EGFR), fibroblast growth factor receptor (FGFR)-4, TNFRSF1b, FLT4, toll-like receptor (TLR)-3, and VEGFA (Table 2). Significant associations (raw P < 0.05, maximum FDR q = 0.051) were observed for 17 SNPs in 8 genes for melanoma-related mortality: 4 SNPs in PDGFD, 3 SNPs in EGRF and IL-10, 2 SNPs in PIK3CA and TLR3, and 1 SNP in FGFR4, FLT1, and LZTS1 (Table 3). Carriers of a minor allele at these SNPs, except for PDGFD rs17423306 and LZTS1 rs2643385, were associated with an increased risk of melanoma-specific mortality.

GWAS data from the MDACC set replicated the result only for rs2220377 in PDGFD (Table 3) with a FDR q = 0.37. Furthermore, we conducted a meta-analysis that combined results across the 2 studies for 4 SNPs (including rs2220377) with ORs in the same direction and P < 0.1 in each study. We identified 3 additional SNPs in the meta-analysis that were significantly associated with SLN metastasis (EGFR rs723526 and TLR3 rs3775292) and melanoma-specific death (TLR3 rs7668666; Fig. 1).

Discussion

This study evaluated the association between 238 SNPs in 26 angiogenesis and lymphangiogenesis pathway genes with SLN metastasis as well as mortality in patients with melanoma. To our knowledge, this is the first study to comprehensively examine genetic variation in the angiogenesis and lymphangiogenesis pathways in relation to melanoma progression. We found suggestive evidence that genetic variants of PDGFD, EGRF, and TLR3 may influence the risk of SLN metastasis and/or death with replication provided for several of the implicated SNPs in a genome-wide scan and meta-analysis.

The mechanism of progression of malignant melanoma cells is still poorly understood. However, it is known that angiogenesis and lymphangiogenesis have been investigated primarily on progression of cancers (15, 16) and play an important role in the progression of melanoma. Previous studies reported that new blood and lymphatic vessels promote the metastatic spread of tumors and
correlated with tumor stage, progression, metastasis, and survival in melanoma (5, 14, 20–27). Therefore, angiogenesis and lymphangiogenesis were suggested as a prognostic indicator for risk for progression of melanoma. A few of the best-characterized genes for these pathways are PDGF, EGFR, and TLR3.

PDGFs are important in proliferation, apoptosis, transformation, migration, invasion, angiogenesis, and metastasis (40). Among 4 PDGF family members, PDGFD is involved in inflammation and angiogenesis (41, 42). Studies have shown that PDGFD is an inducer of transformation in vitro and promoter of tumorigenesis in vitro (43) and that PDGFD stimulates tumor vessel pericyte abundance and enhances tumor growth rate in melanoma (44). No published studies have examined the significance of genetic variation of PDGFD in melanoma. In the current data, a common SNP (rs2220377) in the intron region of the PDGFD gene was associated with an 68% increase in the risk of melanoma-specific death. These results were replicated in the independent MDACC validation set.

Although the intronic rs2220377 variant itself is unlikely to have functional impact, the variant may be in linkage disequilibrium with another SNP that enhances function of PDGFD, creating a milieu favorable to growth and metastasis of tumor cells.

TLRs are involved in acquired immunity and the detection of pathogens including cancer debris (45). Genetic variation in the TLRs has been investigated extensively for association with infectious and noninfectious diseases, including cancer. Recently, Gast and colleagues evaluated 47 SNPs in 8 TLRs for relationships with melanoma susceptibility and survival using 763 melanoma cases and 736 matched controls (46). SNPs in TLR2, TLR3, and TLR4 showed statistically significant differences in the distribution of inferred haplotypes between cases and controls. However, no individual SNP was significantly associated with disease susceptibility. TLR3 is abnormally upregulated on cells isolated from melanoma biopsies and its activation induces melanoma cell migration both in vitro and in vivo (47). In the present study, one SNP in the intron
region of TLR3 (rs3775292) was significantly associated with SLN metastasis and another SNP (rs7668666) with melanoma-specific death, both statistically significant in a meta-analysis combining Moffitt and the MDACC series. The EGFR is a critical protein in proliferation of epithelial cells and is involved in oncogenesis. De Wit and colleagues identified significant differential expression of EGFR in various stages of melanocytic tumor progression, including 19% of nevocellular nevi, 61% of dysplastic nevi, 89% of primary cutaneous melanomas, and 91% of melanoma metastases (48). Furthermore, they observed that staining intensity was stronger in malignant lesions compared with benign lesions (48). These data were supported by recent studies showing overexpression of cytoplasmic EGFR in melanoma as compared with benign nevi (49) and higher expression levels in patients with melanoma with SLN-positive than patients with SLN-negative tumors (50). In the present study, a common variant in the promoter region of the EGFR gene (rs723526) was found to be associated with a significant 2.3-fold increase in risk of SLN metastasis in the meta-analysis.

Associations in several other angiogenesis and lymphangiogenesis genes, including FGFR4, FLT1, and PIK3CA, were identified in Moffitt data consistent with findings of several previous studies (25, 51, 52). However, these associations were not validated in the MDACC series indicating the need for further studies to determine the impact of these genes on melanoma outcome.

The present study benefits from several strengths including a relatively large number of melanoma cases, the selection of genes based on strong biologic rationale, pathologically confirmed cases, and the availability of SLN biopsy results, as well as the availability of clinical and survival data for all cases. Furthermore, we attempted to replicate our results in a recent GWAS of melanoma. Our meta-analytic approach, using a liberal $P$ value less than 0.1 for selecting and then pooling data on SNPs in the discovery and replication datasets, may be explored. Therefore, the identified 2 SNPs (TLR3 rs3775292 and TLR3 rs7668666; Fig. 1) are good candidates that need to be confirmed in future studies. Although we evaluated a comprehensive list of genes in angiogenesis and lymphangiogenesis pathways, our list was not complete, and other genes and SNPs not evaluated in the present study could potentially contribute to SLN metastasis or survival in melanoma.

In conclusion, findings from this exploratory analysis support several previous studies indicating a role for angiogenesis and lymphangiogenesis pathways in regional nodal metastasis and progression of melanoma, and specifically implicate 3 genes (EGFR, PDGF and TLR3) in these processes. Additional studies are warranted to further investigate these findings and to localize potential causal variants.

Disclosure of Potential Conflicts of Interest
J.L. Messina is a consultant/advisory board member of Glaxo Smith Kline. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: J.Y. Park, J.L. Messina, V.K. Sondak, T.A. Sellers, K.M. Egan
Development of methodology: J.Y. Park, K.M. Egan
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.Y. Park, K. Krebs, S. Fang, J.E. Lee, Q. Wei, C.I. Amos, J.L. Messina
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): G.M. Anic, B. Walls, H.Y. Park, K. Krebs, M.H. Madden, K.N. Maddox, S. Marzban, V.K. Sondak, K.M. Egan
Study supervision: V.K. Sondak, K.M. Egan

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node status in cutaneous melanoma: a case-control study. J Clin Pathol
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