Mature MicroRNA Sequence Polymorphism in MIR196A2 Is Associated with Risk and Prognosis of Head and Neck Cancer

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Abstract

**Purpose:** The central role of microRNAs as regulators of translation has been well established, whereas the relationships between genetic variation in microRNAs and disease risk is only beginning to be explored. A polymorphism in the MIR196A2 locus has shown associations with lung, breast, esophageal, and gastric tumors but has not been examined in head and neck cancers, which share similar pathology and etiology to these diseases.

**Experimental Design:** We studied a polymorphism in the mature sequence of MIR196A2 (rs11614913, C/T) in a population-based case-control study (n = 1,039) of head and neck squamous cell carcinoma (HNSCC) to determine if MIR196A2 genotype was associated with disease occurrence and patient survival.

**Results:** Presence of any variant allele was associated with a significantly reduced risk for HNSCC (odds ratio, 0.8; 95% confidence interval, 0.56-0.99). Homozygous variant allele carriers with pharyngeal tumors had significantly reduced survival compared with wild-type and heterozygous cases (hazard ratio, 7.4; 95% confidence interval, 1.9–28.2). Expression analysis in a subset of tumors (n = 83) revealed no significant difference in relative expression of either miR-196a or miR-196a* by MIR196A2 genotype.

**Conclusion:** These data demonstrate a role for MIR196A2 genotype in susceptibility and prognosis of HNSCC.

With ~45,000 new cases diagnosed and >11,000 deaths each year, head and neck squamous cell carcinoma (HNSCC) is a relatively common cancer in the United States (1). Three major etiologic factors contribute to risk and prognosis of HNSCC: tobacco use, alcohol consumption, and human papilloma virus (HPV) infection (2, 3). Recently, examinations of microRNA (miRNA) expression profiles in HNSCC have highlighted the importance of miRNA expression alterations in HNSCC tumorigenesis, disease etiology, and survival (4–10).

miRNAs, short noncoding RNAs that prevent the translation of their target mRNAs, are critical regulators of the transcriptome (11). miRNAs are known to play a crucial role in gene regulation, and altered expression of miRNAs in human cancers has been well documented (12). Single-nucleotide polymorphisms occurring in miRNAs or in their binding sites are novel sources of genetic variation that may contribute to cancer susceptibility and prognosis (13, 14).

A genetic variant in the mature miRNA sequence of MIR196A2 (rs11614913) has been associated with reduced risk for breast cancer (15, 16), gastric cancer (17), and non–small cell lung cancer (18). In addition, this polymorphism has been associated with prognosis in non–small cell lung cancer (19). The MIR196A2 gene encodes two mature miRNAs: miR-196a, which is regarded as the main mature product of the hairpin, and miR-196a*, which contains the single-nucleotide polymorphism rs11614913. Previous investigations have suggested that this single-nucleotide polymorphism affects the processing of the pre-miRNA into its mature, regulatory form (15).

We hypothesize that MIR196A2 genotype is associated with susceptibility to, and/or prognosis of HNSCC, and examined this hypothesis in a population-based case-control study.

**Materials and Methods**

**Study population**

The study population has been previously described (20, 21). Briefly, incident cases of HNSCC were identified from nine medical facilities in the Boston metropolitan...
area, with histologic classification of malignancy reported by pathology of the participating hospitals and confirmed by an independent study pathologist. Population-based controls were drawn from the same greater Boston population and matched to cases by gender, age ($\pm 3$ y), and town of residence using the Massachusetts town lists. All cases and controls enrolled in the study provided written informed consent as approved by the Institutional Review Boards of the participating institutions. Survival time was determined for cases using publicly available databases.

**DNA isolation and genotyping**

DNA was extracted from whole blood or buccal cells with the QIAamp DNA mini kit according to the manufacturer's protocol (Qiagen). Genotyping of the MIR196A2 miRNA single-nucleotide polymorphism (rs11614913, C/T) was done using Taqman allelic discrimination (Applied Biosystems) with a commercially available primer probe set (assay ID C_31185852_10). Genotyping was done in a blinded fashion, appropriate controls were included in each run, and $\sim$10% of samples were duplicated in a coded fashion as quality assurance, with $>95\%$ concordance observed between replicates.

**RNA isolation**

Total RNA was isolated from tumors obtained at initial resection or normal human tissues from head and neck sites obtained from the National Disease Research Interchange that were snap frozen immediately in liquid nitrogen. The mirVANA RNA Isolation Kit (Ambion) was used according to the manufacturer's protocol. RNA was quantified using the Nanodrop ND-1000 spectrophotometer (Nanodrop), aliquoted, and stored at $-80^\circ$C briefly until used for laboratory analysis.

**Quantitative reverse transcription-PCR**

Taqman miRNA Assays (Applied Biosystems) were used to quantify mature miRNAs miR-196a (Custom Assay) and miR-196a* (Assay ID 002336). To test whether the assay for miR-196a differentially amplified target sequence based on genotype, we measured synthetic oligonucleotide sequences for the mature miRNAs and report absolute Ct values because the synthetic miRNA sequences were the only substrate in this experiment. cDNA was synthesized by priming with gene-specific looped primers, including the primers of the miRNAs of interest and RNU44, a universally expressed endogenous control (Applied Biosystems). A 5-$\mu$L volume of total RNA diluted to a final concentration of 10 ng/$\mu$L was used for each reverse transcription reaction along with other reverse transcription components per manufacturer's specifications. Fifteen-microliter reactions were incubated in an Applied Biosystems GeneAmp PCR system 9700 for 30 minutes at 16°C, 30 minutes at 42°C, and 5 minutes at 85°C, and held at 4°C. All reactions, excluding no-template controls and non-reverse transcribed controls, were run in triplicate on an ABI 7500 Fast Real-Time PCR Detection System according to manufacturer's protocol. All real-time PCR data were normalized to the RNU44 transcript (6).

## Statistical analysis

Data were analyzed using the SAS software, and all $P$s represent two-sided statistical tests. Tests for Hardy-Weinberg equilibrium were conducted. Unconditional logistic regression was used to calculate adjusted odds ratios and 95% confidence intervals (95% CI) for the association of MIR196A2 genotype and HNSCC risk adjusting for age, sex, HPV16 status, alcohol consumption, and tobacco-smoking pack-years. Although subjects without HPV16 serology data could have been coded as missing and included, as a more conservative approach, these subjects were not included in the model. For analyses by tumor location, cases were grouped according to International Classification of Diseases-9 code, with oral cancer encompassing 141-145, pharyngeal cancers 146-149, and laryngeal cancers 161. Because homozygous variant and heterozygous genotypes had similar odds ratios, and to improve power for examination of the rare variant allele, these groups were combined, with homozygous wild type (WT) as the reference (dominant model). Patient survival was examined in a recessive model using Cox proportional hazards modeling to control for variables related to patient survival. These survival probability models included variables representing the combined homozygous variant and controlled for patient age (in decades), tumor stage (I and II versus III and IV), and all other covariates in the table. miRNA expression data were subjected to a two-sided, unpaired Student’s t test, assuming equal means and unequal variance.

## Results

Among 1,242 eligible subjects with genotyping data, 203 did not have HPV16 serology data and were excluded. There were no differences in the demographic or exposure characteristics of these individuals compared with the full study population. Characteristics of the remaining 484 cases (269 oral, 123 pharyngeal, and 92 laryngeal cancers)
and 555 controls are described in Table 1 (n = 1,039). Distributions of study participant age, gender, and race between cases and controls were similar. Relationships between disease risk, etiologic factors, and tumor site in this study population have been described previously (3, 20–22).

We examined the association of genotype in the mature sequence of MIR196A2 with case status. Genotypes were within Hardy-Weinberg equilibrium, and the prevalence of control subject genotypes (C/C, 35.3%; C/T, 49.2%; T/T, 15.5%) was nearly equivalent to control subject genotypes reported in another study on subjects predominantly of European descent (C/C, 35.6%; C/T, 49.1%; T/T, 15.3%; ref. 15).

In models controlling for potential confounders, presence of any variant allele was associated with a significantly reduced risk for HNSCC (odds ratio, 0.8; 95% CI, 0.6-0.99; Table 2). The association between the variant allele and decreased risk for HNSCC was evident for individual tumor sites, although only reached statistical significance among cases with pharyngeal disease (odds ratio, 0.6; 95% CI, 0.4-0.96). In overall and tumor site–specific analyses, there was no effect modification of the association between genotype and disease by pack-years smoked, average drinks per week, or HPV16 seropositive status (data not shown).

We then examined the association of the MIR196A2 variant genotype with disease survival. Carriers of any variant allele did not have significantly different survival compared with WT cases (data not shown). In addition, homozygous variant cases did not have significantly different survival probability compared with WT and heterozygous cases (log-rank P = 0.33; n = 418; Fig. 1A). Stratifying by tumor site, there was no significant survival association for cases with oral cavity tumors (P = 0.98; n = 243; Fig. 1B) or laryngeal cancer (P = 0.55; n = 74; Fig. 1C). However, cases with pharyngeal cancer and homozygous variant genotype had significantly reduced survival compared with WT and heterozygous cases (P < 0.02; n = 101; Fig. 1D). Cases with survival data and tumor staging (n = 323) were entered into a multivariate Cox proportional hazards model to control for potential confounders, and no significant association between genotype and survival was found (Table 3). When stratifying the

| Table 1. Participant characteristics of HNSCC cases and controls genotyped for rs11614913 in MIR196A2 |
|--------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Characteristic                                    | All HNSCC sites                | Oral (n = 269)                  | Laryngeal (n = 92)               | Pharyngeal (n = 123)            |
|                                                  | Cases, n (%) (n = 484)          | Controls, n (%) (n = 555)       | Unadjusted OR (95% CI)           | Unadjusted OR (95% CI)          | Unadjusted OR (95% CI)          |
| Age (y), mean (SD)                                | 59.4 (11.6)                    | 61.0 (11.5)                     | 1.0 (Reference)                 | 1.0 (Reference)                | 1.0 (Reference)                |
| Gender                                           |                                 |                                 |                                 |                                 |                                 |
| Male                                             | 359 (74.2)                     | 407 (73.3)                      | 1.2 (0.8-1.9)                   | 1.2 (0.7-2.0)                  | 3.2 (1.0-10.9)                 |
| Female                                           | 125 (25.8)                     | 148 (26.7)                      | 1.6 (1.1-2.2)                   | 1.4 (0.9-2.1)                  | 5.3 (1.9-14.4)                 |
| Race                                             |                                 |                                 |                                 |                                 |                                 |
| Non-White                                        | 41 (8.5)                       | 50 (9.0)                        | 3.3 (2.4-4.5)                   | 2.3 (1.6-3.3)                  | 17.1 (6.7-43.5)                |
| White                                            | 443 (91.5)                     | 505 (91.0)                      |                                |                                |                                |
| Tobacco (lifetime pack-years)*                   |                                 |                                 |                                |                                |                                |
| ≤1                                               | 98 (20.3)                      | 197 (35.5)                      | 1.0 (Reference)                 | 1.0 (Reference)                | 1.0 (Reference)                |
| >1 to ≤8                                         | 43 (8.9)                       | 73 (13.1)                       | 1.2 (0.4-0.8)                   | 0.5 (0.3-0.8)                  | 0.6 (0.3-1.2)                  |
| >8 to ≤34                                        | 111 (22.9)                     | 142 (25.6)                      | 1.6 (0.7-2.0)                   | 0.6 (0.4-0.9)                  | 0.5 (0.2-1.1)                  |
| >34                                              | 232 (47.9)                     | 143 (25.8)                      | 2.3 (1.6-3.3)                   | 1.6 (1.1-2.4)                  | 2.0 (1.2-3.4)                  |
| Alcohol use (lifetime average drinks/wk)*        |                                 |                                 |                                |                                |                                |
| ≤2                                               | 111 (22.9)                     | 122 (22.0)                      | 1.0 (Reference)                 | 1.0 (Reference)                | 1.0 (Reference)                |
| >2 to ≤6                                         | 74 (15.3)                      | 157 (28.3)                      | 0.5 (0.4-0.8)                   | 0.6 (0.3-0.8)                  | 0.6 (0.3-1.2)                  |
| >6 to ≤15                                        | 78 (16.1)                      | 133 (24.0)                      | 0.7 (0.4-0.9)                   | 0.6 (0.4-0.9)                  | 0.5 (0.2-1.1)                  |
| >15                                              | 221 (45.7)                     | 143 (25.8)                      | 1.7 (1.2-2.4)                   | 1.6 (1.1-2.4)                  | 2.0 (1.2-3.4)                  |
| HPV16 seropositivity                             |                                 |                                 |                                |                                |                                |
| No                                               | 341 (70.5)                     | 494 (89.0)                      | 1.0 (Reference)                 | 1.0 (Reference)                | 1.0 (Reference)                |
| Yes                                              | 143 (29.5)                     | 61 (11.0)                       | 3.4 (2.4-4.7)                   | 2.8 (1.9-4.2)                  | 1.8 (1.0-3.3)                  |
| MIR196A2 genotype                                |                                 |                                 |                                |                                |                                |
| C/C                                              | 182 (37.6)                     | 188 (33.9)                      | 1.0 (Reference)                 | 1.0 (Reference)                | 1.0 (Reference)                |
| C/T + T/T                                        | 302 (62.4)                     | 367 (66.1)                      | 0.9 (0.7-1.1)                   | 0.9 (0.7-1.3)                  | 0.8 (0.5-1.3)                  |

Abbreviation: OR, odds ratio.
*Tobacco pack-years and average drinks per week based on quartile distribution in controls.
Table 2. MIR196A2 variant genotype is associated with significantly reduced risk for HNSCC

<table>
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<th>Covariate</th>
<th>All sites Cases, n (%)</th>
<th>Controls, n (%)</th>
<th>Adjusted OR (95% CI) n (%)</th>
<th>Oral Cases, n (%)</th>
<th>Controls, n (%)</th>
<th>Adjusted OR (95% CI) n (%)</th>
<th>Laryngeal Cases, n (%)</th>
<th>Controls, n (%)</th>
<th>Adjusted OR (95% CI) n (%)</th>
<th>Pharyngeal Cases, n (%)</th>
<th>Controls, n (%)</th>
<th>Adjusted OR (95% CI) n (%)</th>
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<td>total n = 555</td>
<td>total n = 269</td>
<td>total n = 92</td>
<td>total n = 123</td>
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<td>32 (10.8)</td>
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<td>84 (31.2)</td>
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<td>367 (66.1)</td>
<td>0.8 (0.6-0.99)</td>
<td>173 (64.3)</td>
<td>0.8 (0.6-1.1)</td>
<td>56 (60.9)</td>
<td>73 (59.3)</td>
<td>0.6 (0.4-0.96)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Models are controlled for all variables.
*Tobacco pack-years and average drinks per week based on quartile distribution in controls.
analysis by tumor site, pharyngeal cancer cases with homozygous variant genotype had significantly reduced survival (hazard ratio, 7.4; 95% CI, 1.9-28.2; Table 3).

We measured expression levels of mature miRNAs miR-196a and miR-196a* in normal head and neck tissues (n = 14) and fresh frozen tumor tissues (n = 83) to determine if MIR196A2 variant genotype affects expression. First, to validate that presence of the variant allele did not interfere with the detection of mature miRNAs, equivalent quantities of synthetic oligonucleotides corresponding to WT and variant miR-196a* sequences (and a 1:1 mix for heterozygous) were measured in triplicate with a stem-loop reverse transcription-PCR assay for miR-196a*. This assay did not differentially amplify targets based on genotype (Ct values: C/C, 15.90; T/T, 16.28; C/T, 16.18). In normal head and neck tissues (n = 14), we found no significant difference in the relative expression of either miR-196a or miR-196a* by MIR196A2 genotype (data not shown). Similarly, in tumor tissues (n = 83), we found no significant difference in relative expression of either miR-196a or miR-196a* by MIR196A2 genotype overall (n = 83) and when stratified by tumor location (Table 4).

**Discussion**

Polymorphisms in miRNA genes and their target sites are a novel class of variation in the human genome that are rapidly being identified and investigated in human cancers, including HNSCC (13, 14, 23–25). Motivated by several recent publications linking the MIR196A2 mature miRNA single-nucleotide polymorphism to cancer susceptibility and prognosis (15–19, 26), we tested the hypothesis that this single-nucleotide polymorphism (rs11614913) is associated with susceptibility to and prognosis of HNSCC; we found a significantly decreased risk for HNSCC, and significantly reduced survival in cases with pharyngeal disease. Notably, the prevalence of control genotypes was completely consistent with another ethnically similar (Caucasian) population-based study (15).

**Fig. 1.** Kaplan-Meier survival probability plots stratified by MIR196A2 genotype for head and neck tumors with available staging data. Survival time is defined as time from diagnosis to death or last known follow-up, where circles represent censored values. The log-rank method was used to test for a difference between strata. Solid lines represent wild-type and heterozygous MIR196A2 genotype cases and dotted lines represent homozygous variant allele cases. A, all tumor sites, n = 418, P = 0.33. B, oral cancers, n = 243, P = 0.98. C, laryngeal cancers, n = 74, P = 0.55. D, pharyngeal cancers, n = 101, P < 0.02.
Table 3. Cox proportional hazards models of overall survival by MIR196A2 genotype controlled for confounders

<table>
<thead>
<tr>
<th>Covariate</th>
<th>All sites Cases, n (%); total n = 323</th>
<th>Oral Cases, n (%); total n = 179</th>
<th>Laryngeal Cases, n (%); total n = 59</th>
<th>Pharyngeal Cases, n (%); total n = 85</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted HR (95% CI)</td>
<td>Adjusted HR (95% CI)</td>
<td>Adjusted HR (95% CI)</td>
<td>Adjusted HR (95% CI)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>77 (23.9)</td>
<td>1.0 (Reference)</td>
<td>9 (15.3)</td>
<td>17 (20.0)</td>
</tr>
<tr>
<td>&gt;50 to ≤60</td>
<td>105 (32.5)</td>
<td>1.2 (0.6-2.4)</td>
<td>23 (39.0)</td>
<td>23 (27.1)</td>
</tr>
<tr>
<td>&gt;60 to ≤70</td>
<td>97 (30.0)</td>
<td>1.9 (1.0-3.6)</td>
<td>18 (30.5)</td>
<td>37 (43.5)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>44 (13.6)</td>
<td>3.2 (1.6-6.5)</td>
<td>9 (15.2)</td>
<td>9 (9.4)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>78 (24.2)</td>
<td>1.0 (Reference)</td>
<td>12 (20.3)</td>
<td>16 (18.8)</td>
</tr>
<tr>
<td>Male</td>
<td>245 (75.8)</td>
<td>1.4 (0.8-2.5)</td>
<td>47 (79.7)</td>
<td>69 (81.2)</td>
</tr>
<tr>
<td>Tobacco (lifetime pack-years)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>64 (19.8)</td>
<td>1.0 (Reference)</td>
<td>4 (6.8)</td>
<td>17 (20.0)</td>
</tr>
<tr>
<td>&gt;1 to ≤8</td>
<td>27 (8.4)</td>
<td>0.2 (0.1-1.1)</td>
<td>3 (5.1)</td>
<td>5 (5.9)</td>
</tr>
<tr>
<td>&gt;8 to ≤34</td>
<td>80 (24.8)</td>
<td>1.0 (0.5-1.9)</td>
<td>16 (27.1)</td>
<td>20 (23.5)</td>
</tr>
<tr>
<td>&gt;34</td>
<td>152 (47.0)</td>
<td>1.1 (0.6-2.0)</td>
<td>36 (61.0)</td>
<td>43 (50.6)</td>
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<tr>
<td>Alcohol use (lifetime average drinks/wk)*</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>78 (24.1)</td>
<td>1.0 (Reference)</td>
<td>19 (32.2)</td>
<td>18 (21.2)</td>
</tr>
<tr>
<td>&gt;2 to ≤6</td>
<td>51 (15.8)</td>
<td>0.6 (0.2-1.3)</td>
<td>10 (17.0)</td>
<td>9 (10.6)</td>
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<tr>
<td>&gt;6 to ≤15</td>
<td>48 (14.9)</td>
<td>0.7 (0.4-1.5)</td>
<td>4 (6.8)</td>
<td>17 (20.0)</td>
</tr>
<tr>
<td>&gt;15</td>
<td>146 (45.2)</td>
<td>1.0 (0.6-1.7)</td>
<td>26 (44.0)</td>
<td>41 (48.2)</td>
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<td>HPV16 seropositivity</td>
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<tr>
<td>No</td>
<td>221 (68.4)</td>
<td>1.0 (Reference)</td>
<td>44 (74.6)</td>
<td>46 (54.1)</td>
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<tr>
<td>Yes</td>
<td>102 (31.6)</td>
<td>0.6 (0.4-1.0)</td>
<td>15 (25.4)</td>
<td>39 (45.9)</td>
</tr>
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<td>Stage</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I or II</td>
<td>93 (28.8)</td>
<td>1.0 (Reference)</td>
<td>25 (42.4)</td>
<td>14 (16.5)</td>
</tr>
<tr>
<td>III or IV</td>
<td>230 (71.2)</td>
<td>2.2 (1.3-3.6)</td>
<td>34 (57.6)</td>
<td>71 (83.5)</td>
</tr>
<tr>
<td>MIR196A2 genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C/C + C/T</td>
<td>275 (85.1)</td>
<td>1.0 (Reference)</td>
<td>49 (83.1)</td>
<td>79 (92.9)</td>
</tr>
<tr>
<td>T/T</td>
<td>48 (14.9)</td>
<td>1.3 (0.8-2.2)</td>
<td>10 (16.9)</td>
<td>6 (7.1)</td>
</tr>
</tbody>
</table>
| NOTE: Models are controlled for all variables. Abbreviation: HR, hazard ratio. *Tobacco pack-years and average drinks per week based on quartile distribution in controls.
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functional relationship between these polymorphisms
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sion in several cancer types and adjacent normal tissues
results. A recent examination of differential miRNA expres-
specific transcriptomes, there are varying etiologic
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comparing expression levels of the mature miR-196a
among MIR196A2 genotypes, Hu et al. (19) observed
significantly lower expression of miR-196a in non–small
cell lung tumor samples with C/C genotype (n = 6) com-
pared to C/T and T/T individuals (n = 17). These authors
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mRNA sequences but did not observe differential expres-
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of the precursor miRNA in their transfection experiment,
suggesting that MIR196A2 genotype may result in altered
processing of the pre-miR (15). Relative to previous work,
our expression results were from a large number of sam-
ple (n = 83), affording sufficient statistical power to detect
even subtle differences, and although the miR-196a ex-
pression results seem to be conflicting, cell type–specific
differential expression of miRNAs could explain these re-
results. A recent examination of differential miRNA expres-
sion in several cancer types and adjacent normal tissues
(including breast and lung, though not head and neck) showed a wide range of miR-196a expression across differ-
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normal tissues from common sites (GSE14985; ref. 27).
If miRNA single-nucleotide polymorphisms directly af-
flect the expression of mature miRNA species, then the
functional relationship between these polymorphisms
and disease susceptibility or prognosis may seem clear: altered miRNA expression leads to altered regulation of
target mRNAs important in tumorigenesis. Nonetheless,
even in the absence of altered mature miRNA expression
levels, variation in the mature miR-196a* sequence itself
could result in differential regulation of target mRNAs.
Mature miR-196a* in variant allele form could have either an increased or decreased affinity for targets of
WT miR-196a*. A determinant of the impact of miRNA
sequence variation is cell type–specific expression levels
of both miRNAs and their targets: cell type–specific transcriptomes. In addition, it is important to note that
tumorigenesis-related alterations in a cell's transcriptome
may also affect the regulatory capacity of miRNAs to the
extent that a miRNA variant allele may have a protective
effect for disease, as well as predict poor prognosis. Func-
tional characterization of miRNA target transcripts is far
from complete, and there is currently little data available
to speculate on cell type–specific regulation of mRNAs by
miR-196a and miR-196a*.

However, unlike some other reports (15, 19), when we as-
essed relative expression of mature miR-196a and miR-
196a*, we did not detect significantly different expression
by MIR196A2 genotype in either normal head and neck
tissues or tumors.

Comparing expression levels of the mature miR-196a
among MIR196A2 genotypes, Hu et al. (19) observed
significantly lower expression of miR-196a in non–small
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tional characterization of miRNA target transcripts is far
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to speculate on cell type–specific regulation of mRNAs by
miR-196a and miR-196a*.

Though there is conflicting data for the association be-
tween MIR196A2 genotype and miR-196a expression, the
variant allele is in the other mature miRNA product of this
gene, miR-196a*. Consistent with our data, Hoffman et al.
(15) did not observe an effect of the variant on expression
of mature miR-196a*. In addition, studies that report
significant associations between the MIR196A2 polymor-
phism and cancer risk [breast, gastric, and non–small cell
lung cancers (in Caucasian and Asian populations)] all show the T allele to be associated with decreased risk for
disease (15–18). Collectively, these results strongly suggest that reduced risk associated with MIR196A2 variant
genotype is not mediated by differential expression of the
mature miRNAs of the gene.

In the context of carcinogenesis, the dynamic relation-
ship between a miRNA and its targets may be modified
by etiologic exposures. Compounded with cell type–specific transcriptomes, there are varying etiologic
exposures associated with different cancers: in HNSCC,
etiolologic exposures may differ between individuals (within tumor site) and are known to have different magnitudes of effect by tumor site. For instance, HPV16-seropositive individuals have a higher risk for developing pharyngeal disease compared with other sites (3). Furthermore, HPV16 and other important etiologic exposures in HNSCC have been associated with specific molecular alterations that may have broader implications for the transcriptome of a cell, and hence may differentially affect regulation by miRNAs. Nonetheless, we did not observe any effect modification by exposures for genotype associations in this study, suggesting that the precise mechanism(s) by which genotype confers risk is too dynamic and complex to fully elucidate. In addition to more complete characterization of the miRNA targets, further study on additional cancers with similar risk factors (e.g., cervical cancer and HPV and lung cancer and tobacco smoking) might also be helpful in illuminating precisely how these variant genotypes confer risk.

This work suggests that patients carrying a variant allele in MIR196A2 have a significantly decreased risk for HNSCC. In addition, homozygous variant cases with pharyngeal disease also had significantly poorer survival, suggesting that the protective effect of the variant allele does not extend to disease severity. Additional studies are warranted to further confirm these findings and explore the relationship between MIR196A2 genotype and risk for other forms of cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

R01CA078609, R01CA100679, and T32ES007272.

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Received 03/15/2010; revised 05/11/2010; accepted 05/14/2010; published OnlineFirst 05/25/2010.

References

Clinical Cancer Research

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Brock C. Christensen, Michele Avissar-Whiting, Lauren G. Ouellet, et al.


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