Development of a multigene classifier for larynx carcinoma

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ABSTRACT

Introduction

Improved prognostic classification of laryngeal carcinomas could help to select patients for conservative therapy or radical surgery thereby increasing the disease-specific survival while preserving function whenever possible. We therefore developed a microarray-based molecular classifier specific for larynx tumors.

Methods

We analyzed the gene expression profiles by means of hybridization to Affymetrix HuGene 1.0 microarrays of 20 laryngeal squamous cell carcinomas (class 2: n = 10) of 11 male patients aged 56-75 years with a preoperative tumor staging IVA and 20 cases (class 1: n = 10) of 11 male patients aged 60-75 years with a preoperative tumor staging IVA, in whom tumors were completely excised at surgery; RMA was performed and arrays were hybridized according to the manufacturer's instructions. Data were normalized following MAA procedure of Bioconductor.

Results

To identify genes differentially expressed between tumors with and without relapse we performed the nearest shrunken centroid classification using 5-fold cross validation of Microarrays (SAM). We obtained a list of 60 classifier genes, among which H19, a maternally imprinted non coding RNA that is involved in the regulation of the insulin-like growth factor signaling and has been described as a tumor suppressor for Wilms tumors, and FOXP2, an imprinted transcription factor whose mutations cause developmental verbal dyspraxia. Both genes are downregulated in tumors that recurred.

Conclusion

Upon independent validation on a larger cohort, the 60-gene prognostic classifier for laryngeal tumors may support the choice of the appropriate therapy, improving might be a hallmark of relapsing laryngeal carcinomas.

PATIENTS AND METHODS

Patients

We analyzed the expression profiles of a series of 40 laryngeal carcinomas, studied in a bank of tumors at the Advanced Molecular Diagnostics Center of Genova (Italy), by means of hybridization to Affymetrix HuGene 1.0 microarrays (Santa Clara, CA) HuGene1.0 arrays following the protocols of the manufacturer, Germany. Informed consent was obtained from all patients. The local hospital committee approved the study.

Tissue microarrays were prepared in the Department of Pathology and Molecular Pathology, University of Genova, Italy. Tissue samples were obtained from formalin-fixed and paraffin-embedded tumor sections of 30 larynx carcinomas (stages IVA) of 10 male patients aged between 56 and 75 years, 5 with and 5 without relapse.

Patients underwent surgery with and without radiotherapy according to the preoperative tumor staging. 3 patients with relapse and 3 patients without relapse underwent to neck dissection and laryngectomy, respectively. The imprinting status of the H19 imprinting domain in the samples was examined by whole genome PCR analysis and methylation analysis.

Gene expression profiling

Tissue material was drawn from formalin-fixed and paraffin-embedded tumors and further used for microarray analysis. Total RNA was isolated by homogenizing in liquid nitrogen, followed by purification on QIAquick columns (Qiagen, Hilden, Germany) and using the RNeasy mini kit (Qiagen). RNA were (reverse transcriptase) transcribed into cDNA and hybridized to Oligo (dT) and HCO24-primer and hybridized to Oligo (dT) and MHO28-primer. Following the protocols.

Microarray Data were normalized following RMA procedure of Bioconductor.

Gene expression patterns were analyzed after hybridization to the HuGene 1.0 microarrays of 20 larynx carcinomas analyzed reveals that all the cases with relapse were correctly identified as high risk cases.

RESULTS

Table 1: Multigene classifier for larynx carcinomas

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene ID</th>
<th>Low Risk</th>
<th>High Risk</th>
<th>Average Risk</th>
<th>Proportion correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>H19</td>
<td>154964_s_at</td>
<td>0.2534</td>
<td>0.0723</td>
<td>0.126</td>
<td>0.71</td>
</tr>
<tr>
<td>IGF2</td>
<td>156396_s_at</td>
<td>0.2534</td>
<td>0.0723</td>
<td>0.126</td>
<td>0.71</td>
</tr>
<tr>
<td>KIF2</td>
<td>156396_s_at</td>
<td>0.2534</td>
<td>0.0723</td>
<td>0.126</td>
<td>0.71</td>
</tr>
<tr>
<td>TPT1</td>
<td>154964_s_at</td>
<td>0.2534</td>
<td>0.0723</td>
<td>0.126</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Table 2: Development of a multigene classifier for larynx carcinomas

<table>
<thead>
<tr>
<th>Classification of Larynx Carcinomas</th>
<th>Low Risk</th>
<th>High Risk</th>
<th>Error Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Relapse</td>
<td>7</td>
<td>3</td>
<td>0.22</td>
</tr>
<tr>
<td>Relapse</td>
<td>0</td>
<td>11</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Overall error rate = 0.09

CONCLUSIONS

We have developed a multigene classifier using genome-wide gene expression data to which we applied a state-of-the-art bioinformatic tool, Prediction Analysis of Microarrays. The software performs a resampling procedure and applies a cross-validation that is particularly suited for relatively small datasets as the one used here. During the development of the classifier, all genes are tested for the predictive potential and the best, non redundant classifier genes are selected iteratively. This procedure has led to the identification of a classifier, containing only four genes, H19, a non coding gene, is the top discriminator. Its expression is clearly lower in cases with tumor recurrence as compared to cases without. Molecular classifiers become more robust when developed on large datasets. However, the classifier determines a strong relative risk of 6.5 and the association between H19 expression and relapse is very strong. We therefore believe that it will resist in further validation studies involving larger numbers of samples. Upon validation, the classifier may be used to classify tumors through its application to biopsies, best as a quantitative PCR assay. Tumors with a low genomic risk can be treated conservatively whereas for tumors with high genomic risk, radical surgery could be offered to the patient or specific high follow-up screenings must be advised.