Overexpression of EphB4, EphrinB2 and EGFR in Papillary Thyroid Carcinoma

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ABSTRACT

INTRODUCTION

• Papillary thyroid carcinoma (PTC) accounts for 70-80% of thyroid malignancies
• No method to predict or diagnose early lymph node involvement in order to guide targeted treatment
• Molecular mechanisms involved in PTC invasion and lymph node spread: 1) Epidermal growth factor receptors (EGFR) 2) Eph receptors and their ligands
• Molecular crosstalk between EGFR and Eph signaling noted in HNSCC may play a role in PTC tumorigenesis and spread as well.

Our objective is to identify key molecular constituents in the pathogenesis and spread of PTC, so that these biomarkers may be used as early predictors of lymph node involvement and potential therapeutic targets.

MATERIALS & METHODS

Twenty-one (21) adult patients with PTC underwent total thyroidectomy and level VI nodal dissection at a tertiary-level hospital setting.

cDNA Microarray

Four samples (stage III) of matched tumor and normal tissue were randomly selected. mRNA was isolated from fresh tissue sections by standard methods [6]. Human universal RNA was used as a common reference for all experiments. The mRNA was then processed and analyzed via GeneChip U133 Plus 2.0 array (Affymetrix Inc.) against 47,500 genes. Raw data were imported to microarray database (mAdb) and analyzed by software tools provided by the Center for Information Technology, NIH.

Western blot

Western blot was performed on all 21 samples of matched tumor and normal tissue. Cell lysates were prepared as previously described [7]. β-actin was used as a loading control, and was measured for all genes.

Immunohistochemistry

Immunohistochemistry (IHC) analysis was conducted on all 21 samples of matched tumor and normal tissue.

RESULTS

Patient Characteristics

• cDNA: differential expression of EphB4, EphrinB2, EGFR in tumor vs normal tissue (fold change ≥1.5)
• Western blot: EphrinB2, EphB4 and EGFR higher expression in tumor vs normal
• IHC: EphB4 expression statistically significant association between tumor and LN invasion, ECS; EphrinB2 expression statistically significant association between tumor and LN invasion; EGFR expression statistically significant with clinical stage

Table 1. cDNA Microarray analysis. \( P \)-value from two-tailed test

<table>
<thead>
<tr>
<th>Protein name</th>
<th>Gene Symbol</th>
<th>Fold change</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eph receptor B4</td>
<td>EPHB4</td>
<td>2.49</td>
<td>.004</td>
</tr>
<tr>
<td>Ephrin B2</td>
<td>EFNB2</td>
<td>2.42</td>
<td>.001</td>
</tr>
<tr>
<td>Epidermal growth factor receptor</td>
<td>EGFR</td>
<td>2.86</td>
<td>.003</td>
</tr>
</tbody>
</table>

Table 2. Immunohistochemistry analysis. log2 fold change of IOD data. \( P \)-value from Student’s paired t-test (linear regression model adjusted for race).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold change (SE)</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EphB4</td>
<td>-3.93 (0.14)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>EphrinB2</td>
<td>-3.66 (0.15)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>EGFR</td>
<td>-2.47 (0.07)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

CONCLUSION

Elevated expression of EphB4, EphrinB2 and EGFR in PTC compared to normal tissue.

Association between lymph node disease and EphB4 and EphrinB2 expression in tumor.

Molecular markers involved in lymph node metastasis of PTC at a microscopic level; potential targets for directed therapy.

REFERENCES

8. Table 3. Multivariate linear regression analysis (IHC).