Immunohistochemical Analysis of HB-EGF Expression in Breast, Colon, Gastric, Hepatocellular, Lung, Ovarian, Pancreatic and Prostate Cancer

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Introduction and Methods

HB-EGF expression has been demonstrated in many tumor types, and therapeutic monoclonal antibodies are currently under investigation. The current study was performed to develop an immunohistochemical assay for HB-EGF detection and evaluate expression in multiple cancers. The following tests were performed for acceptable performance:

- R&D Systems, Cat# AF-259-NA, goat IgG, polyclonal
- R&D Systems, Cat# MAB2591, mouse IgG, clone 406316
- Cosmo Bio, Cat# BAM 71-501, mouse IgG, clone 4G10
- Santa Cruz, Cat# sc-4143, goat IgG, polyclonal
- U3 Pharma, 2.12.1-biotinylated human IgG2, clone U3-M
- U3 Pharma, 1.19.3-biotinylated human IgG2, clone U3-L

Antigen retrieval and antibody titration studies were performed to optimize staining performance, and acceptable antibodies were used to stain characterized xenografts, characterized cell lines, placenta (HB-EGF-positive) and/or ovarian cancer. The AF-259-NA goat polyclonal antibody demonstrated staining that matched expectations in the characterized samples and was selected for evaluation of cancer and normal tissue samples. The following cancer samples were stained: bladder, breast, colon, gastric, hepatocellular, lung, ovarian, pancreatic and prostate cancer. Normal bladder, breast, colon, liver, lung, placenta, prostate, ovary, skin and stomach were also stained. The inter-day assay precision, or reproducibility, was characterized by staining 3 samples over 5 days and the %CV was calculated.

Results

Comparison of HB-EGF Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>H-score</th>
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</thead>
<tbody>
<tr>
<td>EFO-27 HB-EGF Cell Line</td>
<td>2.12.1</td>
</tr>
<tr>
<td>EFO-27 HB-EGF Xeno</td>
<td>1.19.3</td>
</tr>
<tr>
<td>MD-A-MB-231 Xeno</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Figure 1: Immunohistochemistry staining results of optimized IHC assays applied to characterized samples. Optimal antigen retrieval methods were identified for six antibodies against HB-EGF. A titration analysis was performed on the EFO-27 cell line. HB-EGF transformed EFO-27 xenografts were stained, along with the untransformed EFO-27 cell line, MD-A-MB-231 and NCI-H508 cell lines. Only the AF-259-NA antibody detected high levels of expression in the HB-EGF transformed cells, while displaying weak to moderate staining in the low expression controls.

Figure 2: Images of select cancer samples.

Figure 3: Immunohistochemical staining results were evaluated by a pathologist, and the percentage of cells staining 3+ (strong), 2+ (moderate), 1+ (weak) or negative was recorded. Immunohistochemical results are represented graphically using an H-score. The H-score is calculated as the product of staining intensity (1-3) and the percentage of stained cells divided by 100, ranging from 0% to 100%. The percentage of cells stained at each intensity is also graphed, although staining may be present in <1% of cells.

Figure 4: Images of select normal samples. Images of two stomach samples are displayed to demonstrate staining heterogeneity.

Figure 5: Inter-day H-Score precision was evaluated by staining 3 samples on 5 days. The average %CV was 11.7%.

Conclusions

- An HB-EGF IHC assay using the AF-259-NA goat polyclonal antibody was developed and demonstrated proper staining of characterized controls that matched the expected HB-EGF subcellular localization of cytoplasmic, membrane and extracellular.
- Hepatocellular, gastric and ovarian cancer demonstrated the greatest expression of HB-EGF by immunohistochemistry.
- The HB-EGF IHC assay demonstrated acceptable sensitivity, specificity and reproducibility for evaluation of clinical trial samples.