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

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Abstract

Heparin-binding EGF-like growth factor (HB-EGF) is a member of the epidermal growth factor (EGF) family of proteins. It is synthesized as a transmembrane protein, which can be cleaved at the cell surface to release the soluble ectodomain sHB-EGF. HB-EGF has been implicated in several physiological and pathological processes, including tumorigenesis and metastasis. Anti-HB-EGF therapeutic monoclonal antibodies are currently under investigation and hold promise for treating multiple types of cancer. In order to investigate the relationship between HB-EGF expression in cancer and response to therapy, an immunohistochemical assay to HB-EGF was developed and validated. Antigen retrieval and antibody titration studies were performed for six antibodies in order to select an antibody with proper performance characteristics. The antibodies were tested in characterized xenografts, characterized cell lines, placenta (HB-EGF-positive) and/or ovarian cancer. Of the antibodies tested, the AF-259-NA goat polyclonal antibody demonstrated the appropriate staining pattern in xenografts, cell lines and placenta. The optimized immunohistochemistry assay was used to stain cancer samples, and the frequency of moderate (2+) or strong (3+) staining in cancer cells was as follows: gastric = 50%, hepatocellular = 50%, lung = 40%; ovarian = 38%, colon = 37%, breast-ER+ = 20%, bladder = 10%; breast-HER2+ = 10%, pancreatic = 10%, breast-triple negative = 0%, prostate = 0%. When compared by average H-score, the cancer ranked in the following order (H-score in parenthesis): Hepatocellular (69), ovarian (37), gastric (23), breast-HER2+ (14), breast-ER+ (12), pancreatic (10), lung (5), breast-triple negative (2), colon (2), bladder (1), prostate (1). For comparison, H-scores for normal tissues were as follows: Normal bladder epithelium (74), stomach (24), colon (8), prostate (3), ovary (1), liver (1), breast (0), lung (0) and epidermis (0). In summary, immunohistochemistry using the AF-259-NA antibody produces the expected staining pattern in characterized samples and can be applied to human cancer samples to evaluate the relationship between clinical response and HB-EGF expression.

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 antibodies were tested 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-1413, 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

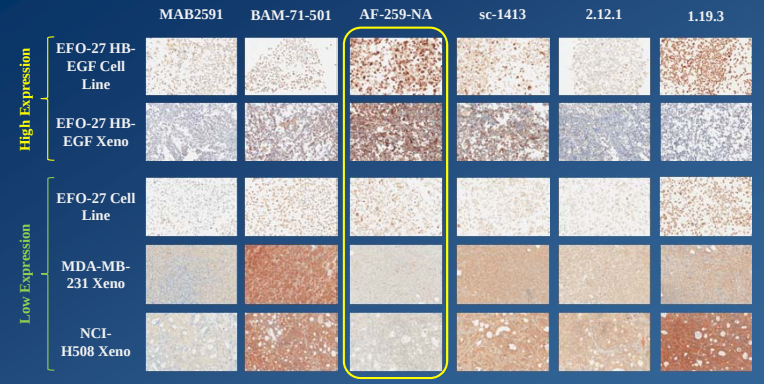

Figure 1: Immunohistochemistry staining results of optimized IHC assays applied to characterized samples. Optimal antigen retrieval methods were identified for six antibodies against HG-EGF. A titration analysis was performed on the EFO-27 cell line, HB-EGF transformed EFO-27 cell line, and xenografts from the HB-EGF transformed EFO-27 cell line, MDA-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.

HB-EGF (AF-259-NA) Staining of Select Cancer Samples

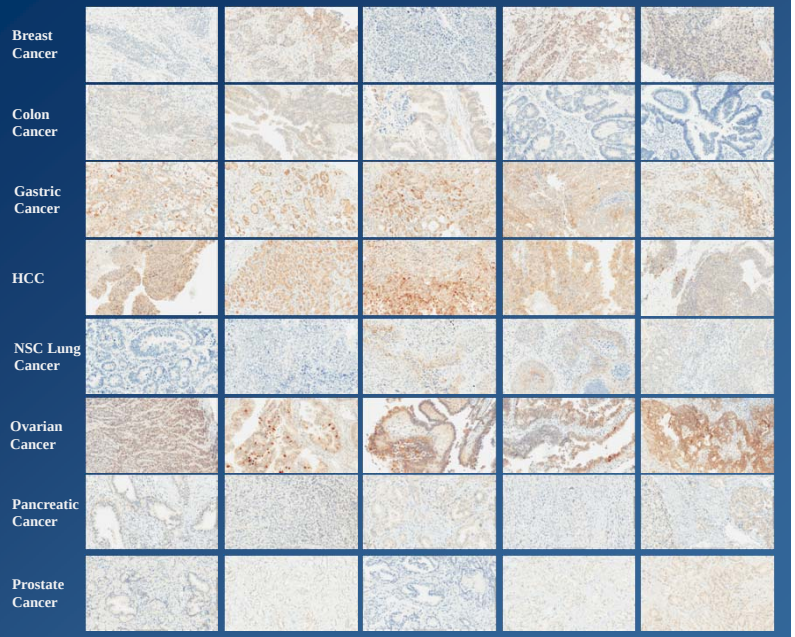

Figure 2: Images of select cancer samples.

HB-EGF Expression in Cancer and Normal Tissues

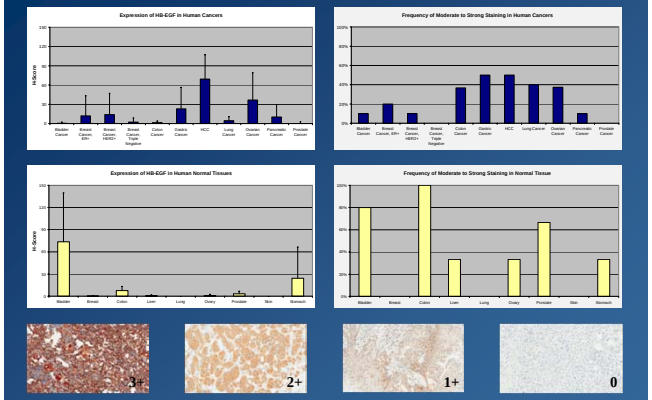

Figure 3: Immunohistochemical staining results were evaluated by a pathologist, and the percentage of cells staining 3+ (strong), 2+ (moderate), 1+ (weak) or negative was recorded. Immunohistochemical results are represented quantitatively using an H-score. The H-score is calculated as the summation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

HB-EGF (AF-259-NA) Staining of Select Normal Tissues

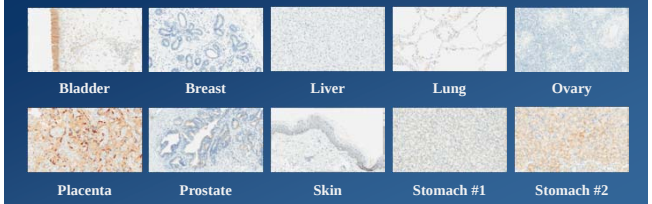

Figure 4: Images of select normal samples. Images of two stomach samples are displayed to demonstrate staining heterogeneity.

Assay Reproducibility

Specimen ID	Tissue Type	Mean	SD	%CV
MPB01091	Placenta	58	6.48	11.17%
ML0907130	Xenograft	278	21.6	7.77%
ML0907131	Xenograft	70.4	8.35	11.87%
			Average	10.27%

Figure 5: Inter-day H-Score precision was evaluated by staining 3 samples on 5 days. The average %CV was 10.27%.

Conclusions

- An HB-EGF IHC assay using the AF-259-NA goat polyclonal antibody was developed and demonstrated proper staining of characterized controls and 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.