EDB+FN is an attractive target in oncology: Insights from protein expression analysis of solid tumors

Sara Lewandowski[1], Liyang Diao[1], Alyssa Quigley[1], Marsha Crochiere[1], Jan Pinkas[1]

[1]Pyxis Oncology, Boston, MA

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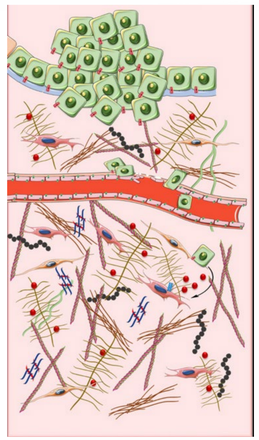
Background



- Stroma plays a major role in the initiation, growth, survival, and drug-resistance of solid tumors, yet few therapeutics specifically target tumor-associated stroma.
- Extra-domain B splice variant of fibronectin (EDB+FN) is an splice variant of fibronectin, a matrix protein upregulated in solid tumor stroma, which is associated with tumor growth, angiogenesis, and metastases.
- Here an immunohistochemistry (IHC) assay was developed to assess EDB+FN protein expression in the stroma of tumor and normal human tissues to characterize the potential of EDB+FN as a therapeutic target for solid tumors with high unmet need.

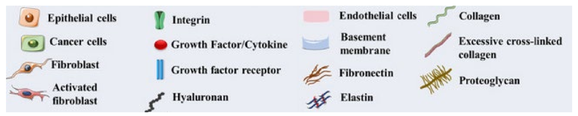


Figure 1: Tumor induced stroma is comprised of multiple cellular and extracellular matrix components, including fibronectin (1).

Methods

- An IHC assay for detection of EDB+FN protein expression in formalin-fixed paraffin-embedded (FFPE) tissues was developed. Anti-EDB+FN monoclonal antibody (clone L19), specific for detection of only the FN1 splice variant that contains extra-domain B, was titrated for a wide dynamic range of detection of weak, moderate, and strong expression (1.5 µg/mL, Fig. 2).
- To evaluate EDB+FN expression patterns and prevalence across cancer indications, whole tissue slides were commercially sourced and stained following an optimized IHC protocol. Slides from approximately 20 individuals per indication were evaluated.
- EDB+FN IHC staining was scored by a pathologist using an H-score approach, separately scoring EDB+FN expression in three areas: tumor-induced stroma, tumor cell membrane, and tumor cell cytoplasm.
  - H = (1 x % area, level 1) + (2 x % area, level 2) + (3 x % area, level 3)
- Percent stroma, by area, within each tumor tissue was determined by a pathologist using H&E-stained serial sections.
- A digital pathology algorithm was developed for streamlined assessment of EDB+FN expression in preclinical models. Briefly, using Qupath software, a tumor/stroma classifier was trained with multiple examples of tumor and stroma morphology across a range of indications and this classifier was applied to categorize individual cells within each slide scan. Thresholds for level 1, level 2, and level 3 expression intensity were set using pathologist-scored samples as a guide. Combined areas of each cell category and each intensity threshold are used to compute percent stroma and EDB+FN digital H-score, respectively.

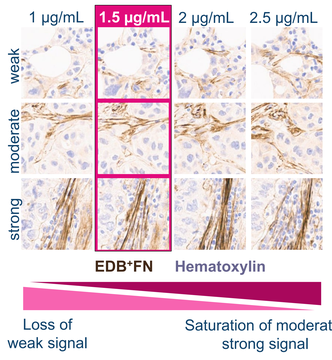


Figure 2: IHC using antibody clone L19 at a concentration of 1.5 µg/mL shows a wide dynamic range of detection for EDB+FN protein expression in FFPE tissue sections of lung cancer.

EDB+FN is broadly and predominantly expressed in tumor-induced stroma across multiple cancer indications

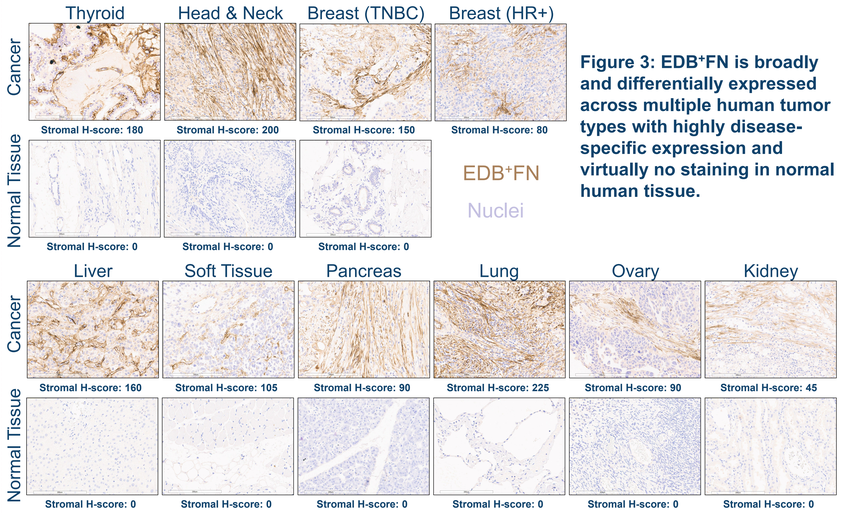
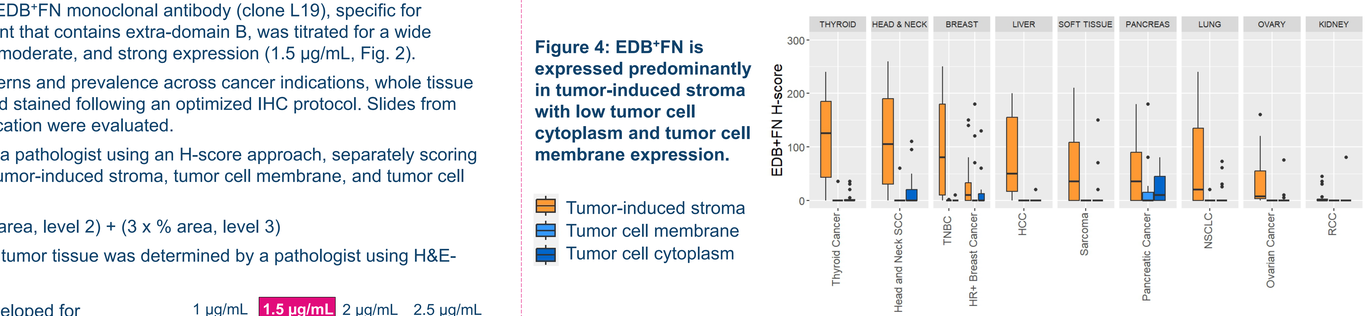


Figure 3: EDB+FN is broadly and differentially expressed across multiple human tumor types with highly disease-specific expression and virtually no staining in normal human tissue.

Figure 4: EDB+FN is expressed predominantly in tumor-induced stroma with low tumor cell cytoplasm and tumor cell membrane expression.



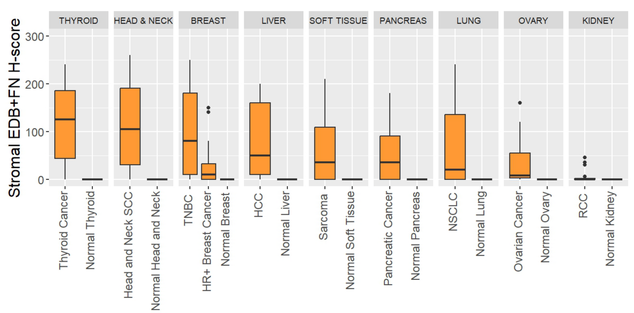


Figure 5: EDB+FN exhibits disease-specific expression in tumor induced stroma, with little to no expression in normal tissue stroma.

Amount of stroma is not itself predictive of EDB+FN expression

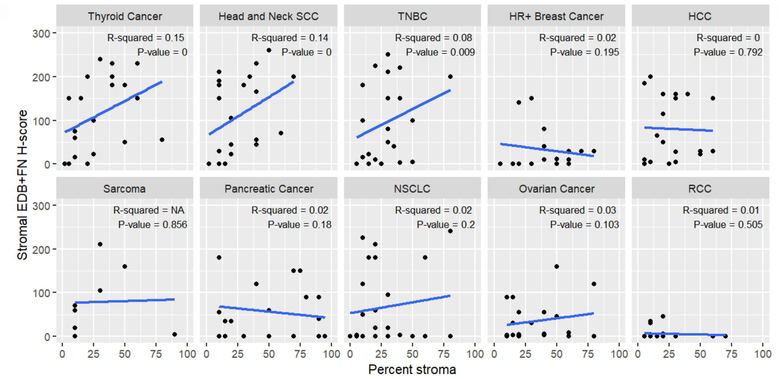


Figure 6: Stromal EDB+FN expression does not strongly correlate with percent stroma. Y-axis shows stromal EDB+FN expression score based on IHC and X-axis shows percent stroma from serial section H&E. Each point represents one individual (some points may be overlapping).

Digital pathology algorithm developed for streamlined scoring of EDB+FN IHC

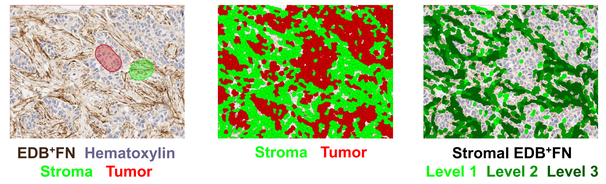


Figure 7: Development of a digital pathology algorithm for scoring EDB+FN IHC. A classifier was trained using examples of tumor cell (red) and tumor-induced stroma (green) morphologies from multiple indications and applied to categorize cells. Thresholding was applied with intensity cutoffs for each EDB+FN expression level set using pathologist scores as guides. Combined areas of each cell category and each intensity threshold were used to compute percent stroma and EDB+FN digital H-scores, respectively. This algorithm can be used for streamlined scoring of preclinical samples.

Conclusions

- The tumor-induced, stroma-specific expression of EDB+FN and broad distribution of expression across indications make EDB+FN an ideal target for high-unmet-need solid tumors.
- Percent stroma itself is not predictive of EDB+FN expression within a tumor, underscoring the need for a specific and robust assay to evaluate EDB+FN protein expression.
- The specificity and dynamic range of the IHC assay developed by PYXS for detection of EDB+FN protein expression position it as a robust potential biomarker assay for EDB+FN-targeting therapeutics.
- Work is ongoing using digital pathology to evaluate the distribution of EDB+FN expression and potential correlations with tissue architecture and cancer pathology.

References

(1) Jurj, A., Ionescu, C., Berindan-Neagoe, I., & C. Braicu (2022). The extracellular matrix alteration, implication in modulation of drug resistance mechanism: friends or foes? Journal of Experimental & Clinical Cancer Research, 41, 276.

Contact Info: Presenting & Primary Author Sara Lewandowski slewandowski@pyxisoncology.com