ED-B Fibronectin as a potential marker for tumor microenvironment & fibrosis

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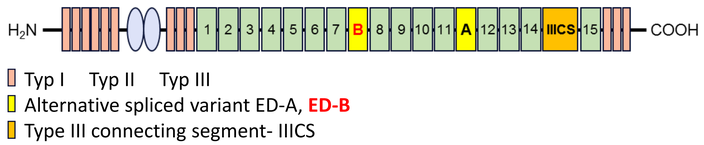
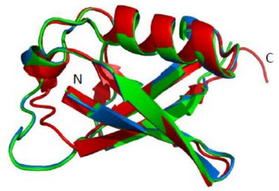
Objective

The **glycoprotein fibronectin** (FN) is involved in tissue remodeling and is one of the lead cancer-related extracellular matrix proteins within the tumor microenvironment. Its alternative spliced variant ED-B FN is described as an oncofetal protein and is almost absent in healthy adult tissue.



Typ I Typ II Typ III
Alternative spliced variant ED-A, **ED-B**
Type III connecting segment- IIICS

During pathological processes such as **tumorigenesis** and **fibrogenesis**, ED-B FN can be re-expressed. Hence, imaging of ED-B FN may be of great interest for early detection and therapy monitoring of fibrotic and oncological diseases.
Therefore, we analyzed various tumor and fibrotic samples with **Affilin®-77405**, which is a ubiquitin based scaffold and can be used as an alternative to antibodies for multiple applications (IHC, Western blotting, as biomarker, etc).



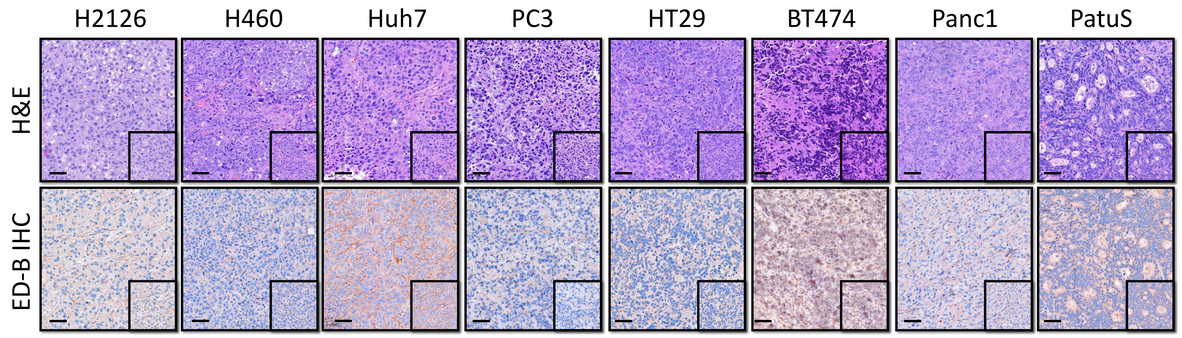
N- & C-terminal domain overlay of Affilin®-77405 (blue and green) and ubiquitin (red).

Methods

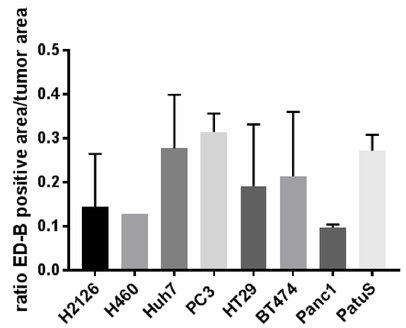
IHC with Affilin®-77405 was performed on **xenografts** (BT474, H2126, HT29, H460, Huh7, Panc1, PatuS, PC3), a **transgenic lung fibrosis mouse model** (Fra-2, 20 weeks old), **a bleomycin induced lung fibrosis mouse model** (BLM, 14 days after exposure to BLM) and on **human lung tissue** (donor, IPF). Lung tissue from wild-type or sham-treated mice served as control. Quantification of ED-B FN staining was done via an automated imaging analysis software (StrataQuest).

Results

1. Xenografts

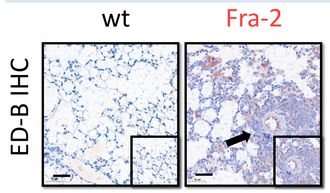


Representative images of H&E (top) and ED-B IHC (bottom) stainings on the different Xenograft samples. Scale bar 50µm.

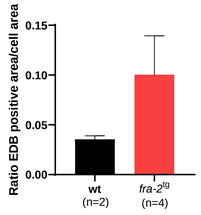


n≥3 for each tumor

2. Transgenic mouse model

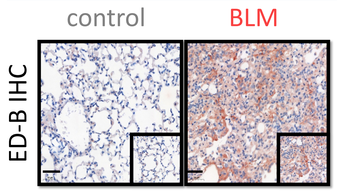


Representative images of ED-B IHC from 20 weeks old wt and Fra-2 lungs.
Scale bar 50µm.

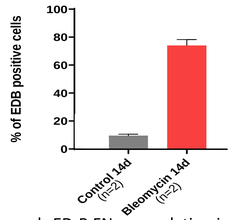


ED-B was not significantly but on trend upregulated in Fra-2 lungs compared to wt lungs.

3. Bleomycin mouse model

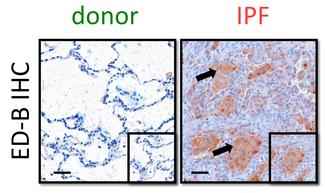


Representative images of ED-B IHC from BLM- and sham-treated (14 days) mice..
Scale bar 50µm.

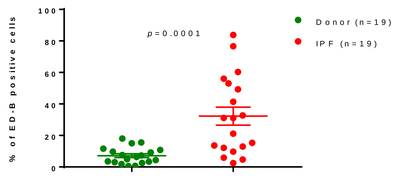


Clear trend towards ED-B FN upregulation in bleomycin model.

4. Human lungs



Representative images of ED-B IHC from donor and IPF human lungs.
Scale bar 50µm.



ED-B was highly significantly upregulated in IPF lungs compared to donor lungs.

Conclusion

As ED-B FN is almost absent in healthy adult tissue it may represent an attractive target for imaging and therapy of cancer and fibrosis.

Affilin®-77405 evinced to be suitable for detection of ED-B FN in these diseases.

Furthermore, Affilin®-77405 can be labelled with different radionuclides making it suitable for non-invasive *in vivo* imaging and for potential therapy.