

Fibroblast Subtype Composition at Diagnosis Improves Progression Risk Estimates in Non-Muscle-Invasive Bladder Cancer

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Introduction: About 75% of bladder cancers are non-muscle-invasive at diagnosis, and the central clinical problem is predicting which will progress. Guideline risk groups that guide the choice between intravesical BCG and cystectomy are built from tumor features alone. Cancer-associated fibroblasts (CAFs) are heterogeneous, but whether CAF subtype composition adds prognostic information in non-muscle-invasive disease has not been tested against a progression endpoint.

Methods: We integrated five published single-cell RNA sequencing datasets into an atlas of 315,628 cells from 34 treatment-naïve tumor samples (26 patients) and resolved 5,950 fibroblasts into four subtypes, separating fibroblasts from smooth muscle cells and pericytes. We characterized each subtype by matrix gene expression and compared subtype shares between tumor and normal bladder by spatial transcriptomics (four tumor sections, one normal section). We estimated subtype fractions in bulk RNA sequencing from an independent cohort of 530 treatment-naïve patients (UROMOL) by Bayesian deconvolution. We applied log-ratio transforms for Cox modeling and measured added value for progression-free survival as the change in Harrell's C-index, tested by likelihood ratio.

Results: Two subtypes had opposite associations with progression. The dominant collagen and extracellular matrix producer was associated with higher risk. This subtype expressed ACTA2 (alpha-SMA) in 67.9% of cells, yet ACTA2 separated smooth muscle cells more strongly than it separated this subtype (detection difference 0.700 against 0.467). The same subtype made up 2.7% of fibroblasts in normal bladder against 24.7 to 67.6% across tumor sections. A second subtype was associated with lower risk. Adding this protective subtype to a clinical model of age, sex, smoking and clinical risk score raised the C-index from 0.772 to 0.796 (likelihood ratio $p=4.4e-04$, 1 degree of freedom). Adding total stromal cell abundance instead raised it to 0.774 ($p=0.151$).

Conclusions: Fibroblast subtype composition adds prognostic information to clinical risk factors in non-muscle-invasive bladder cancer, and in this model it outperforms total stromal abundance. The adverse subtype is enriched in tumor relative to normal bladder, and alpha-SMA alone does not distinguish it from smooth muscle. Subtype fractions can be estimated from bulk sequencing of diagnostic tissue, without an additional biopsy.