98 Single cell flow cytometry and machine learning stratifies patients undergoing transurethral resection of the bladder tumor (TURBT)

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Introduction & Objectives: Bladder cancer (BCa) is the most common malignancy of the urinary tract with an estimated a high rate of recurrence. 70-80% of cases are non-muscle invasive bladder cancer (NMIBC) at diagnosis of which 60-70% will recur and 45% of high-risk patients will progress to muscle invasive bladder cancer (MIBC) in five years. Emerging evidence in the literature firmly associates the presence of diverse cancer stem cell (CSC) populations to tumor initiation, progression and treatment failure[1]. The aim of this study is to perform unsupervised analysis of BCa cells in NIMBC patient samples and identify a high-risk biomarker using a novel machine learning algorithm.

Materials & Methods: Pre-, mid- and post-surgical washouts and urine samples were collected from BCa patients who underwent TURBT in the past two years. Single-cell suspension was prepared following mechanical and enzymatic dissociation. Flow cytometry analysis of these cells was done using a panel of commercially available monoclonal antibodies against lineage markers: CD45/CD31/CD326, published BCa CSC markers: CD90/CD44/CD49f[2] and other markers representing tumor initiation, inflammatory, angiogenesis and epithelial mesenchymal transition (EMT) signatures: CD24/CD44[3] as well as other markers: CD47, MHC-I (both self-markers) CD317(metastasis) and CD73 (mesenchymal) plus live/dead cell discrimination. Finally, the relationship of all cellular populations is visualized in 2D space and their presence/absence is statistically evaluated using clustering/embedding algorithm EmbedSOM[4]. We have performed hierarchical dissection of viable cells of known lineages down to the identification of unique rare cell clusters which were positive for a combination of cell surface markers.

Results: Patient clinical history, cytology and histology assessment were correlated with the presence or absence of a particular BCa population. Two one-sided Mann-Whitney tests were used to evaluate the presence or absence of a particular cell population between low-risk and high-risk patients as well as between controls, non-recurrent and recurrent/progressing patients. Our results confirm very high heterogeneity of BCa phenotype with a few statistically significant cell populations in high-risk patients.

Conclusions: A combination of single-cell flow cytometry with machine learning algorithm identifies unique tumor cell populations in both TURBT washouts and urine samples as a promising biomarker for future non-invasive stratification of high-risk BCa patients. Functional characterization of these populations must be however validated in a xenotransplantation model.

References:

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