Transcriptional signatures provide alternative views of mCRPC pathological subtypes

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Background: During tumor evolution, differentiation and transdifferentiation may give rise to transitional cancer types that reside between well-established subtypes. In prostate cancer, some adenocarcinomas develop neuroendocrine characteristics and eventually transition into small cell neuroendocrine carcinoma, a rare and highly aggressive subtype. Using RNA-seq to classify such transitional states and characterize genomic events driving the transition is crucial for understanding the progression of advanced prostate cancer.

Methods and Results: Unsupervised clustering of RNA-seq data from 183 mCRPC samples revealed four dominant clusters, including one central cluster and three peripheral clusters. Several samples were distributed on "spokes" connecting the central cluster to peripheral clusters, suggesting that these samples represent different steps in a related continuous process or that a linear mixing gives rise to intermediate-positioned samples. Differential gene expression and pathway enrichment analyses suggested three peripheral clusters corresponding to 1) small cell-like disease, 2) metastasis originating in the liver, and 3) dedifferentiation. Interestingly, epithelial-to-mesenchymal–implicated genes had expression changes distinguishing between central versus dedifferentiation clusters.

Pathology review categorized samples into small cell, adenocarcinoma, a newly identified "intermediate atypical carcinoma" (IAC) subtype, or a mixture of these three classes. Clusters had a mixture of pathology subtypes and samples were distributed amongst the RNA-seq clusters. In line with previous work, supervised classification using linear classification (LC) was able to distinguish small cell from adenocarcinoma with a cross-validation accuracy of 84%. Three-way LC placed the IAC samples on a continuum between the small cell and adenocarcinoma samples. Supervised trajectory inference provided a pseudotemporal ordering of samples along transitional axes, revealing potential associated gene regulatory programs. VIPER master regulator analysis revealed several distinct regulators for IAC, including GATA2, a known driver of prostate cancer aggressiveness, NR4A1 associated with recurrence in NSCLC1, and MAFK, which promotes EMT and progression in triple-negative breast cancer.

Conclusions: Unsupervised analysis supports the notion that intermediate samples residing between clusters represent snapshots along a disease continuum, such as progression to a more pluripotent state in this case. Pathology-based subtypes had weak correspondence to the unsupervised clusters, with only one cluster showing significant enrichment for small cell disease. Yet an accurate LC was obtained, suggesting that expression-based changes underlie differences in small cell versus adenocarcinoma, but represent more subtle signals that need to be teased out with supervision. In addition, LC confirmed the pathology-derived IAC definitions revealing the samples as an intermediate subtype between adenocarcinoma and small cell with a confidence estimate of $p<10^{-14}$. Master regulator analysis of IAC found many regulators shared with the other two subtypes as expected, but also several with predicted activity specific to IAC. These regulators could represent important biomarkers signifying an early change from adenocarcinoma to the more aggressive small cell subtype.

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