Cell-Free Chromatin Profiling as a Non-Invasive Predictor of Treatment Response in Head and Neck Cancer

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Background: Predicting treatment response in head and neck cancer (HNC) prior to therapy initiation remains a major clinical challenge. Most liquid biopsy approaches target tumor-derived DNA and are insensitive to the immune microenvironment, which plays a critical role in treatment outcome.

Method: We profiled pre-treatment plasma from 25 HNC patients (17 complete responders [CR], 8 progressive disease [PD]) and 100 healthy controls using cell-free chromatin immunoprecipitation sequencing (cfChIP-seq), capturing chromatin accessibility across nearly 20,000 genes. Gene set enrichment, transcription factor activity inference, and causal network modeling were used to characterize the immune and regulatory programs distinguishing responders from non-responders. cfChIP-seq findings were evaluated alongside complementary plasma-based liquid biopsy modalities using unsupervised clustering and regularized feature selection to assess relative predictive contribution.

Results: cfChIP-seq revealed two distinct inflammatory programs separating PD from CR patients: a graded core-inflammatory signature (IL-6/JAK/STAT3, TNF-α/NF-κB) enriched progressively from healthy controls through CR to PD, and a PD-specific interferon program (IFN-γ, IFN-α) absent in CR versus healthy comparisons. Causal network analysis identified a binary regulatory switch, with IRF1, STAT1, STAT2, and SPI1 activated in PD, and HNF4A, HNF1A, and NCOA1 enriched in CR. Unsupervised clustering achieved complete separation of PD from CR (Fisher $p < 0.001$), and cfChIP-seq emerged as the dominant predictive modality among the liquid biopsy data types assessed.

Conclusion: Pre-treatment cfChIP-seq is a promising non-invasive tool for treatment response stratification in HNC, capturing distinct immune programs rather than a generic cancer signal. These findings support prospective validation of cfChIP-seq — potentially integrated with additional plasma-based modalities — as a pre-treatment biomarker platform, and nominate the IRF1/STAT1 axis as a candidate therapeutic target.