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Comprehensive circulating tumor DNA and CTC profiling of treatment naïve early-stage head and neck cancer patients reveals early signature of disease progression

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BACKGROUND

Head and Neck (HNC) carcinoma remains a substantial global health burden due to rising incidence rate in Southeast Asia.

Circulating tumor DNA (ctDNA) and Circulating tumor cells (CTCs) are an emerging biomarker for HNC cancer precision diagnostics and effective targeted therapy. However, ctDNA has not been explored in HNCs primarily originating due to tobacco habits. Exploring genomic profiling to determine mutational drivers for disease progression, therapy relapse and detecting early clinical determinant is an unmet need and may help in theragnostic as well as prognostic applications.

PROBLEM STATEMENT

Although clinically considered as a locoregional disease, biologically HNC shows systemic traits, and hence detecting genomic parameters, and disease progression indicators (CTCs) may help determining disease status and therapy response. Comprehensive genomic drivers of HNCs induced due to carcinogenic hits (e.g. tobacco habits) still remains unknown and hence it is therapeutically significant to detect actionable target for better management.

METHODS

ctDNA and CTCs of eighteen, early-stage HNC patients were detected and analyzed for genomic landscapes using the Illumina NextSeq 2000 next generation sequencing platform with paired end read (2 x 150 bp). A custom-designed OncoIndx gene panel was used for the hybrid capture target-enrichment of critical cancer genes (600). This in-house OncoIndx panel was designed to detect frequently mutated genes of functional relevance in cancer targeting the exonic sequence of 600 genes reporting SNVs and indels along translocations and copy number amplification. Additionally, the gene panel detected genome-wide signatures including bTMB (blood tumor mutation burden), MSI (microsatellite instability), HRD (homologous recombination deficiency) prediction and calculate cfDNA tumor fraction. The raw sequencing data was analyzed using In house developed iCare® platform. Variant calling was performed according to AMP/ACMG and ClinVar guidelines.

FIGURES

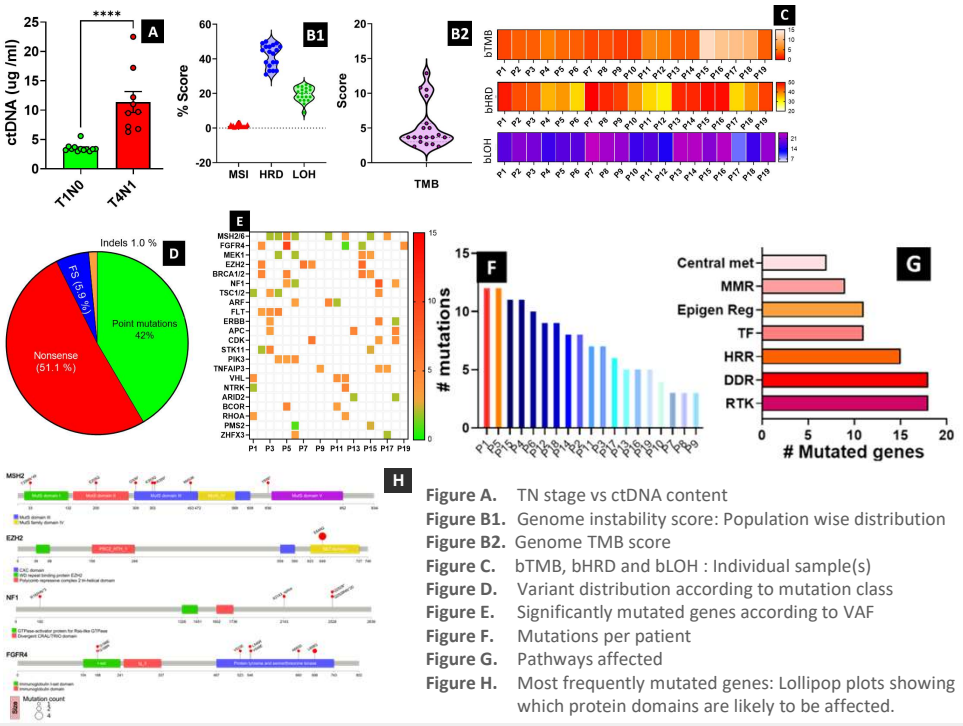

Figure A. TN stage vs ctDNA content
Figure B1. Genome instability score: Population wise distribution
Figure B2. Genome TMB score
Figure C. bTMB, bHRD and bLOH : Individual sample(s)
Figure D. Variant distribution according to mutation class
Figure E. Significantly mutated genes according to VAF
Figure F. Mutations per patient
Figure G. Pathways affected
Figure H. Most frequently mutated genes: Lollipop plots showing which protein domains are likely to be affected.

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RESULTS

- CTCs were detected in peripheral blood from 80% of the localized HNC patients. Comprehensive plasma cfDNA profile of early-stage HNC cancer patients predominantly had low bTMB and MSI Scores (99% patients).
- HRD and LOH matrix was high for 60% patients indicating highly dysregulated DNA repair activities.
- Concurring to these observations, 98% patients had mutations in key tumor suppressor (TS) and DNA damage response (DDR) genes possibly resulting in their loss of function.
- About 65% patients showed paired mutations with DDR / mismatch repair and TS genes governing cell division and growth.
- Besides DNA damage pathway, 60% patients harboured alterations in RTK genes including FGFR, EGFR and PDGFR family and 32% patients showed activating mutations in Erk1 and its upstream regulators.
- MSH family genes were the most prominently mutated (45%) followed by FGFR (33%). Surprisingly, unlike HPV positive advanced HNC cases, TP53 mutations were not detected in any patient, though alterations in TS genes were most prevalent in the study population. 51.1% alterations were nonsense, possibly contemplating to truncated proteins, while 42% alterations were point mutations, 5.9% were frameshift and 1% indels. Presence of mutations in BRAF, PDGFR, FGFR and KIT genes suggested for the potential therapy resistance besides endowing growth advantage to cancer cells. Tumor fraction representing ctDNA showed elevated range from 20% to 45% with a corresponding ploidy between 2 to 4.

CONCLUSIONS

- Comprehensive ctDNA profile revealed major variant from TS and DDR response pathway, besides mutations in proliferative signaling proteins. TP53 mutation was virtually absent, although variants such as RB and RAD family members were detected at low frequency. This suggests for a unique mutation pattern associated with early-stage HNC due to tobacco etiology.
- Presence of pathogenic or potential pathogenic mutations in DDR and proliferative pathway strongly indicated for the novel therapeutic possibility with either an off label or clinical trial options in primary HNC patients. Our results suggest that comprehensive ctDNA analysis along CTC profiling can predict the disease progression beforehand, and may offer new treatment options to early-stage HNC patients.