NEOANTIGENS LANDSCAPE IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

LUCIANA RODRIGUES CARVALHO BARROS[1], PAULO THIAGO DE SOUZA SANTOS[1], MARCO ANTONIO MARQUES PRETTI[1], GUSTAVO FIORAVANTI VIEIRA[2,3], MARCELO ALVES DE SOUZA BRAGATTE[3], MARCUS FABIANO DE ALMEIDA MENDES[3], MARTIELA VAZ DE FREITAS[3], NICOLE DE MIRANDA SCHERER[4], IVANIR MARTINS[5], DAVY CARLOS MENDES RAPOSO[5], TATIANA DE ALMEIDA SIMÃO[6], SHEILA COELHO SOARES LIMA[1], MARIANA BORONI[4], LUIS FELIPE RIBEIRO PINTO[1,6], MARTIN HERNAN BONAMINO[1,7]

1. Programa de Carcinogênese Molecular, Instituto Nacional de Câncer - INCA, Rio de Janeiro, Brazil.
2. Programa de Pós-Graduação em Saúde e Desenvolvimento Humano/Universidade La Salle-Canoas
3. Programa de Pós-Graduação em Genética e Biologia Molecular/UFRGS
4. Laboratório de Bioinformática e Biologia Computacional, Instituto Nacional de Câncer, INCA
5. Divisão de Patologia, Instituto Nacional de Câncer, Rio de Janeiro, Brazil.
6. Departamento de Bioquímica, IBRAG, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.
7. Vice-Presidência de Pesquisa e Coleções Biológicas (VPPCB) - Fundação Oswaldo Cruz, FIOCRUZ.

INTRODUCTION

Esophageal cancer (EC) is one of the ten most incident and lethal neoplasms worldwide. The chemotherapy of choice still involves taxane and platinum-based regimens, without any molecular targets. Therefore, it is of utmost importance to better characterize these tumors in order to develop biomarkers and new therapeutical strategies. Esophageal squamous cell carcinoma (ESCC) exhibits high intratumoral molecular heterogeneity that might favor immunotherapy, such as the immune checkpoints blockade. Nonetheless, the success of such therapies depends on the immune based microenvironment characteristics of the tumor.

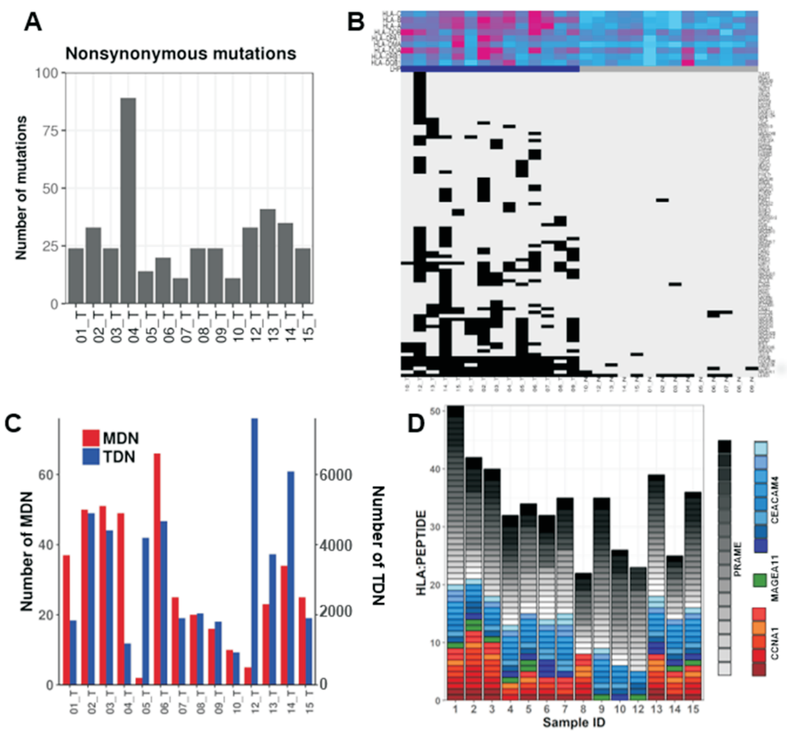
METHODS

Objective: Describe the immune cell infiltrate in ESCC tumor, find prognosis biomarkers and propose new treatment strategies to ESCC patients.

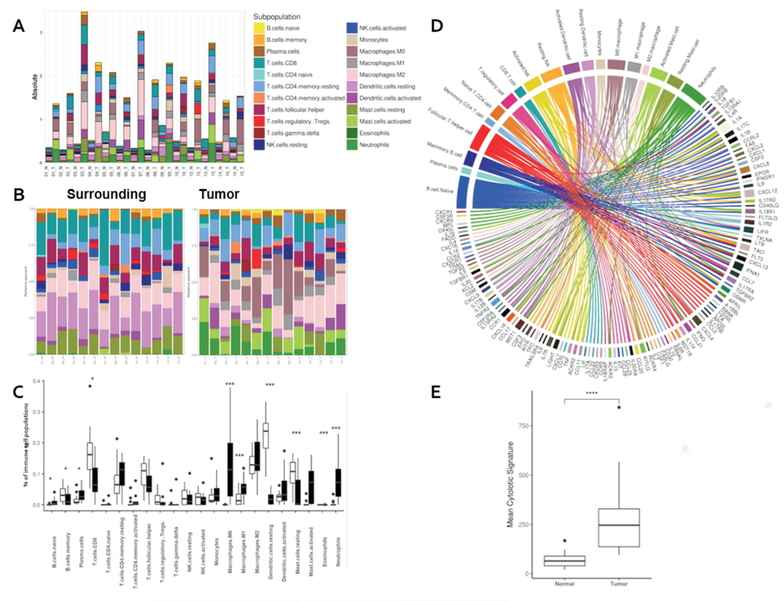
RNA-seq from 14 tumor and adjacent normal tissue samples from ESCC patients without previous treatment (from BNT, INCA - CEP 116/11) were performed by Illumina Hi-Seq 2000. Mutations were analyzed by GATK best protocols. Neoantigens were predicted based on NetMHCpan 3.30 and HLA alleles by Optitype. CIBERSORT was used to calculate 22 immune cell subpopulations percentage. MiXCR and tcR package was applied to estimate TCR and BCR clonotypes. R packages were used for graphs and statistics.

RESULTS

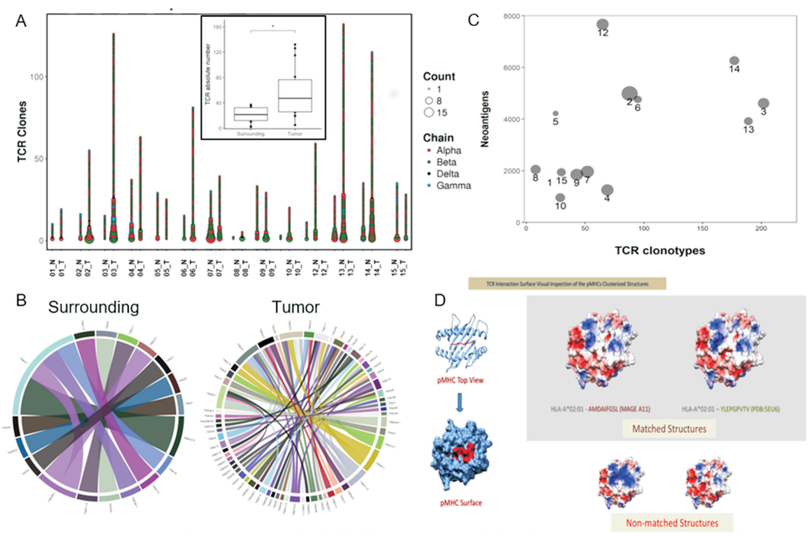
We found 75 mutations per sample (32-206)(**A**)**.** Based on 4-digits HLA-A, -B and -C alleles of each patient, we found 29 neoantigens in average (2-66) per sample (red bars). Thousands of neoantigens were derived from TAA for each sample (924-7606) (blue bars)(**B-C**). Tissue associated antigens (TAA) expression was evaluated revealing four genes expressed by most tumor samples with no expression in normal tissue. The expression of class I and II HLA genes were higher than normal tissue (right panel)(**D**).



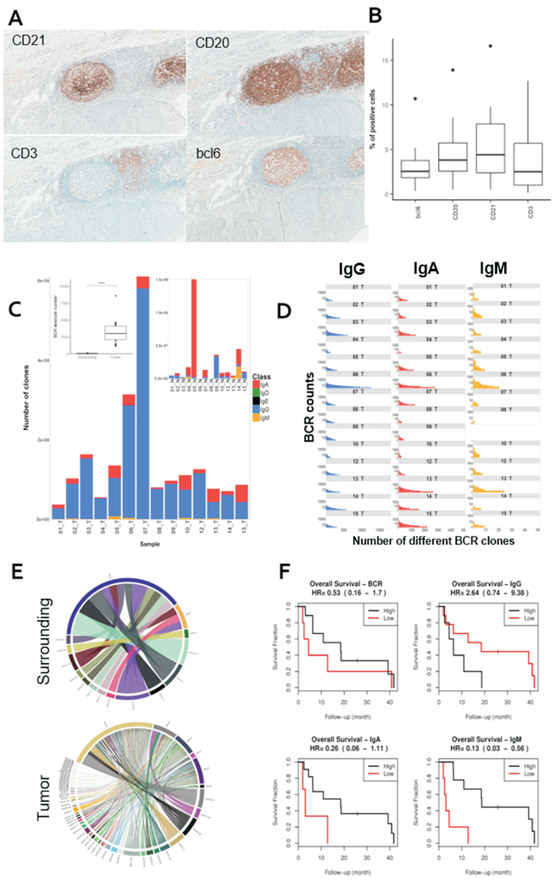
Of 22 immune cell subpopulations evaluated by immune signature deconvolution analysis T CD8, memory B cells, M0/M1 macrophages, mast cells, and dendritic cells, eosinophils (**A-C**). Several cytokines were also correlated with mostly B cells populations and all others (**D**). B memory cells confers a protective prognosis in our and TCGA-ESCA cohorts.



Tumor samples presented more variety and expansion of TCR clones, being alpha and beta chains the most abundant, with no VDJ segments usage preference (**A**). Cristal structures of a peptide derived from MAGEA11 presented a very similar structure of the same TCR CDR3 sequence founded in Protein Databank (PDB) **(B).**



Tertiary lymphoid structures were found in half of ESCC patients (**A-B**). Tumors have more BCR than the surrounding tissue (**C - insert**). BCR repertoire were predominantly IgG whereas IgA was in surrounding area (**C**). Expanded clones were found within ESCC tumors (**D-E**). IgM clone numbers confer a better prognostic marker for ESCC (**F**).



CONCUSION

We described the complex role of several immune cell subsets, its inhibitory and stimulatory molecules in mucosa collaborating for the tumor microenvironment. Also, we found a TCR specific for a neoantigen. Altogether these results showed that each patient has its own collection of neoantigens derived from mutations, but shared peptides from TAA genes that can be proposed as a unique peptide vaccine for ESCC patients

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