IDENTIFICATION AND FUNCTIONAL VALIDATION OF NEOANTIGENS-SPECIFIC T-CELL RECEPTORS IN LYNCH SYNDROME

Fahriye Duzagac*, Krishna Sinha, Ana Bolivar, Nan Deng, Laura Reyes Urbe, Alexandre Reuben, Eduardo Villar-Sanchez. MD-Anderson Cancer Center, Houston, TX, USA

Background Lynch syndrome (LS) is the most common cause of hereditary colorectal cancer (CRC), with >1 million estimated carriers in the US. Normal cells in LS patients become MMR-deficient (MMRd) after acquiring a somatic ‘second hit’ in the same MMR gene, thus generating thousands of small insertion/deletion (indels) mutations. Indels occurring in coding microsatellite regions give rise to immunogenic frameshift peptides (FSPs) that become mutated neoantigens (neoAg). LS elicits a distinct immune response directed against FSP neoantigens stimulating the adaptive immune cells through recognition by T cell receptors (TCR) of cytotoxic CD8+ T cells. Despite the recent advances in silico prediction algorithms for identifying candidate neoAg peptides, large-scale validation of these peptides is still challenging due to low frequency of Neoag-specific T cells and limited availability of patient samples. Our objective was to characterize neoAg-specific T cells response in LS carriers and to validate the functionality of recurrent and shared mutated neoantigens-specific T cells and their TCRs.

Methods We have identified and validated recurrent and shared MHC-I restricted mutated neoAg peptides in a cohort of LS patients diagnosed with pre-cancer and colorectal cancers using an enzyme-linked immunospot (ELISPOT) assay. NeoAgs validated for eliciting in vitro immunogenicity were used to prime autologous naïve T cells from healthy donors. NeoAg-specific T cells were detected and isolated using Flex-T™ HLA-A*02:01 UVX biotinylated monomers derived peptide-MHC (pMHC) multimers staining. Cytotoxicity and infiltration of isolated NeoAg-specific T cells were measured using innovative NeoAg-specific T cell-mediated co-culture assays in a physiologically relevant microfluidic setup. NeoAgs that showed high cytotoxic activity were used for downstream single-cell TCR sequencing (10x Genomics).

Results We used healthy donor T cell repertoires to eliminate the hurdle in identifying immunogenic and reactive NeoAg. We have validated a set of novel recurrent NeoAg such as RNF43 that showed in vitro immunogenicity with a threshold >30 SFU in ELISPOT assay. Then, we identified and isolated Neoag-specific T cells that recognize endogenously processed and presented epitopes using peptide-MHC (pMHC) multimer staining. Our results showed 5 to 8% CD8+ NeoAg specific T cells enrichment. Robustly enriched and isolated T cells showed cytotoxicity against LS patient-derived organoids that expressed determined NeoAgs. We profiled TCR sequences of validated NeoAg with single-cell TCR sequencing that will serve to perform immune monitoring and early cancer detection.

Conclusions This data suggest that identified neoepitopes and corresponding TCRs provide robust candidate biomarkers to track immunogenicity after vaccination and TCR-based immune monitoring for LS carriers.

REFERENCES

Ethics Approval NeoAgs were identified using paired whole-exome sequencing (WES) and mRNAseq in 10 LS CRC (stage I-III) and 33 precancers (7 advanced adenomas and 26 adenomas). A total of 43 patient samples were recruited during their routine screening colonoscopy to an IRB-approved biopsy specimen protocol (MDACC IRB# PA12-0327).