IDENTIFYING NEOANTIGENS IN LUNG ADENOCARCINOMA PATIENTS

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Background Identification of neoepitopes/neoantigens represents a novel approach to cancer immunotherapy. In this study, we identified and characterized potential neoantigen peptides from lung adenocarcinoma patient and tested their immunogenicity to induce anti-tumor activity.

Methods Six patient samples were examined. The tumor tissue from the first patient was used for whole exome sequencing and bulk RNA sequencing. Most potential candidate peptides were selected based on the surface markers Ly9 (CD229), PLVAP, MBOAT7 and non-surface molecules TLN1, ABLIM3, DAXX, DST and neurofascin. As a result, we synthesized 3 peptides with each bearing a single amino acid mutation (Mu peptides) and 3 of their respectively paired wild type (WT) peptides. We cultured tumor-infiltrating lymphocytes (TILs) from tumor tissue and isolated CD3+ T cells and CD19+ B cells from PBMCs collected from the patients. The CD3^-CD19^- PBMCs were used to generate antigen presenting dendritic cells (DCs).

Results In cytotoxicity (CTL) assays to evaluate the anti-tumor effect, we found that DCs presenting mutant peptides could stimulate autologous TILs and PBMC CD3+ T cells to kill tumor cells significantly more than DCs presenting WT peptides. We also observed direct killing of autologous tumor cells by PBMC CD19+ B cells stimulated with DCs presenting mutant peptides. Importantly, autologous effector TILs, T cells, B cells stimulated with mutant peptide-DCs demonstrated specific killing of tumor cells vs. normal tissue cells used as controls. Pemetrexed (PEM) pre-treatment sensitized cancer cells to cytotoxic killing. In addition to CTL activity against tumor, DCs presenting mutant peptides polarized the autologous TILs and PBMC CD3+ T cells towards a Type 1 profile by secreting significantly higher levels of IFNg and TNF, but lower level of IL-6 in response to tumor cells than DCs presenting WT peptides. Furthermore, DCs presented mutant peptide to autologous PBMC CD19+ B cells, resulting in higher levels of IgG and IgM production in response to tumor cells than DCs presenting WT peptide.

Conclusions We assessed 3 mutant peptides identified from lung cancer patient and evaluated their immunogenicity as evident by their capacity to elicit tumor-specific cytotoxicity and cytokine secretion as well as antibody production by autologous TILs, PBMC T cells and B cells respectively. Mutant neopeptides provoked a proinflammatory response and simultaneously downregulated immune suppressive responses. These mutant peptide(s)-activated/expanded immune cells for adoptive transfer in xenograft animal models will demonstrate their anti-tumor efficacy in vivo. In addition, chemotherapy, e.g., pemetrexed may condition tumors to neoepitope-based immunotherapy.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0139