142 DETECTION OF KRAS G12D/G12V NEOEPITOPES EXPRESSED BY CANCER CELL LINES USING LC-MS/MS

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Background The targeting of mutated neoantigens with T-cell receptor (TCR) based therapies represents a promising strategy to treat cancer. Proof of concept for this approach recently was demonstrated in a patient with metastatic pancreatic cancer who experienced a clinical response after infusion of T cells engineered to express TCRs targeting KRAS G12D neoepitopes.[1]

However, TCR therapy is limited only to patients who express the specific human leukocyte antigen (HLA) molecule that presents a specific neoepitope to the TCR. While there are a number of bioinformatic algorithms that can predict whether a peptide will bind to a given HLA molecule, not all neoepitopes predicted to bind HLA are naturally processed and presented by tumor cells.

The successful deployment of TCR-based therapies requires knowledge of whether the targeted neoepitope is naturally presented by tumor cells. To this end, we developed a system that allows for the identification of peptides that are bound by HLA-I molecules expressed by cancer cell lines expressing KRAS mutations.

Methods We selected 12 cancer cell lines that contain KRAS G12D or G12V mutations and express a variety of HLA alleles. The HLA class I peptides were analyzed by microLC-Triplem Quadrupole MS (Mikros-LCMS-8060, Shimadzu Corporation) and FAIMS-Lumos (Thermo Fisher). The multiple reaction monitoring (MRM) methods for detection of candidate neoantigens were optimized using the authentic synthetic peptides The potential binding capacity of the candidate neoepitopes to each allele were calculated using the NetMHCpan4.1 algorithm.

Results Using our developed MRM analysis, we detected three KRAS G12D mutation-derived peptides and three KRAS G12V mutation-derived peptides that were naturally processed and presented by cancer cell lines expressing endogenous levels of KRAS antigen and HLA. One of these peptides was a novel KRAS G12D neoepitope that was detected by both MRM and data-dependent acquisition.

Conclusions Our platform validated the expression of previously identified KRAS neoepitopes and importantly was able to identify a novel KRAS G12D neoepitope that is presented by cancer cells.

We are currently determining whether TCRs targeting this novel KRAS G12D neoepitope can be found in patients with cancer, which if successful, could lead to the development of a new TCR therapy against this neoepitope. In addition, we have begun to evaluate whether other driver mutation-derived neoepitopes can be identified using the same approach. The discovery of novel neoepitopes derived from oncogenic driver mutations ultimately will expand the number of eligible patients that can be treated with promising TCR-based therapies.

REFERENCE

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