150 CLASS I AND II NEOANTIGEN MAPPING IN MSI-HIGH COLORECTAL CANCER IN NEEDLE BIOPSY SIZE TISSUE SAMPLES

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Background Colorectal cancer (CRC) is currently the second most common cause of cancer related mortality in the world. One of the hallmarks of CRC is its complex mutational landscape that contributes to the derivation of neoantigens which aid the immunosurveillance of the cancer. Immunopeptides play an essential role in adaptive immunity by activating and ensuring T-cell specificity. Mass spectrometry (MS) is currently the only technology that can measure and identify the immunopeptide profiles of biological samples at a large scale, however, MS-based studies are frequently limited by sample input and poor scalability. Here, we introduce a semi-automated workflow requiring low sample input to robustly identify immunopeptides and apply it to a cohort of fresh frozen, high quality CRC samples for immunopeptide profiling and neoantigen identification.

Methods We initially optimized a workflow that allowed the native lysis and sequential immunoprecipitation of class-I and class-II immunopeptides while ensuring scalability and reproducibility. The immunopeptides were thereafter subjected to our TrueDiscovery FAIMS-DIA LC-MS/MS platform. We utilized data from WGS on both tumor and adjacent normal tissue for the calling of high-confidence somatic variations and the definition of the resulting neoantigens.

Results We characterized 131,578 unique immunopeptides from 15 mg of fresh-frozen tumor tissue across 20 patients with CRC lesions that mapped to 12,488 genes. On average we identified 9,767 class-I and 16,445 class-II immunopeptides from all patients indicating significant inter-patient heterogeneity. Overall, we found the identification numbers of class-II immunopeptides to be more variable than that of class-I which might be linked to immune infiltration levels.

From our pool of identified immunopeptides, we were able to detect 16 class-I and 29 class-II neoantigens, covering 87% of the microsatellite instability-high (MSI-H) samples in the cohort. Coverage of both classes was essential to increase neoantigen discovery as 53% MSI-H samples had neoantigens mapping to a single class. Interestingly we also detected a significant correlation between the mutational burden and the number of detected neoantigens in our patient cohort suggesting that the genetic set up of the cancer might influence the repertoire of presented neoantigens thereby controlling immunosurveillance.

Conclusions In summary, we have established a scalable and efficient pipeline for cell line and tissue immunopeptidomics for both class-I and II immunopeptides. Our pipeline generates high-quality identifications and can be deployed to help shed light on (neo)antigen heterogeneity through large-scale profiling of patients as exemplified in the case of MSI-H CRC.

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