912 PREFERENTIAL RECOGNITION OF NEOANTIGENS OVER NON-CANONICAL PEPTIDES IN CANCER PATIENTS

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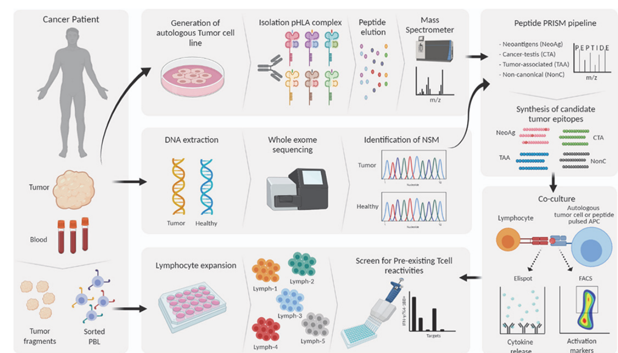
Background Despite recent advances in exome and RNA sequencing to identify tumor-rejection antigens including neoantigens, the existing techniques fail to identify the vast majority of antigens targeted by tumor-reactive cells. A growing number of studies suggest that HLA-I peptides derived from non-canonical (nonC) open reading frames or derived from allegedly non-coding regions can contribute to tumor immunogenicity. Here we use proteogenomics to identify personalized candidate canonical and non-canonical tumor-rejection antigens and to evaluate their contribution to cancer immune surveillance in patients.

Methods Whole exome sequencing was performed to identify the non-synonymous somatic mutations (NSM) and immunopeptidomics to identify the HLA-I presented peptides (pHLA) in 9 patient-derived tumor cell lines (TCL). Peptid-PRISM proteogenomics pipeline was used to identify both canonical and non-canonical pHLA, including those derived from NSM in coding regions. All peptides containing mutations and derived from either cancer-testis (CTA) or tumor-associated antigens (TAA) were selected as candidate tumor antigens. For nonC peptides, an immunopeptidomics healthy dataset containing several tissues and HLA-allotypes was used to eliminate those derived from normal ORFs and select nonC peptides preferentially expressed in tumor cells (nonC-TE). The selected candidate peptides were synthesized, pulsed onto autologous APCs and co-cultured with tumor-reactive ex vivo expanded lymphocytes to assess immune recognition (figure 1).

Results NonC-TE peptides were identified in all TCL studied, ranging from 0.5% to 5.4% of the total HLA-I presented peptides (n= 506). As described previoulsy, 5'UTR were the main source. Of note, the tumor type did not have an impact on the frequency of presented nonC peptides, but rather the presence of HLA-A*11:01 and HLA-A*03:01 was a major determinant. T cell responses were detected against at least 13/33 putative neoantigens, 2/24 CTA and 2/61 TAA. On the contrary, none of the 471 nonC-TE candidate peptides tested thus far, including one containing a NSM were able to elicit a recall immune response. Nevertheless, T cells recognizing at least 3 of them were detected through in vitro sensitization of non-autologous PBMCs.



Abstract 912 Figure 1 Workflow diagram

Tumor biopsies and blood samples are obtained from cancer patients (left panel). Patient-derived tumor cell lines are generated in vitro, the peptides presented on HLA molecules are further isolated and analyzed in a mass-spectrometer (top panel). Whole exome sequencing (WES) from matched tumor and healthy tissue is performed to identify the non-synonymous somatic mutations (NSM) (middle panel). Peptide-PRISM proteogenomics pipeline combines the information from the immunopeptidomics data and WES to identify pHLA sequences from both canonical and non-canonical candidate tumor antigens (top right panel). Lymphocyte populations either TILs or sorted PBMCs are expanded and further screened for pre-existing T cell responses (bottom panel) against the candidate epitopes by co-culturing the T cells with peptide-pulsed autologous APC. The recognition is assessed by measuring IFNg release by elispot and the upregulation of activation surface markers by FACS (bottom right panel).

Conclusions Our results show that although HLA-I nonC peptides were frequently presented in all TCLs studied and they can be immunogenic, neoantigens derived from mutations in canonical coding regions were preferentially recognized by tumor-reactive lymphocytes, suggesting T cells targeting the latter are primed more efficiently. The identification of mutated nonC antigens using whole genome sequencing to identify mutations in non-coding regions warrants further examination. Still, the specificity of many tumor-reactive TILs remains unknown.

Ethics Approval "This study was approved by the "Comité de Ética de Investigación con Medicamentos del Hospital Universitario Vall d'Hebron" institution's Ethics Board; approval number PR(AG)537/2019."

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