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 non-coding regions were preferentially recognized by tumor-reactive cells (nonC-TA) 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 previously, 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.

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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