Immunopeptidome Profiling of Xenograft Glioma Samples

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KEY FINDING

Depletion of mouse MHC complexes prior to IP shows minimal contribution of mouse MHC-associated peptides to human immunopeptidome in xenograft samples.

ABSTRACT

Characterization of the peptides presented by MHC molecules (pMHC) on cancer cells and their immunogenic potential is key for generating anti-cancer immune responses. Experimental identification of pMHC is performed using MHC immunoprecipitation and mass spec sequencing of eluted peptides. Due to sample complexity and material requirements for mass spec, relatively large sample sizes are required for these immunopeptidome identification experiments. This can present a problem for patient-derived material and cells which are difficult to grow in culture. Patient-derived xenograft (PDX) and cell line-derived xenograft (CDX) may represent an attractive methodology to acquire more biologically relevant material for these intensive experiments. In the current study, we characterize the immunopeptidome of two human glioma lines, U87 and DIPG, which were grown in nude mice. As one potential complication of cells grown in mice may be contribution of mouse pMHC to the overall peptide list, we tested specific depletion of mouse MHC prior to immunoprecipitation of human MHC complexes. We analyzed all peptide lists for the actual contribution of mouse-derived peptides to the immunopeptidome and found the true contribution of murine peptides to be minimal.

METHOD

Glioma cells (1x10^6 U87 or 5x10^6 SU-DIPG13P*) were implanted into the flank of 6-week-old nude female mice. Once tumors reached a volume of 100 mm^3, as assessed by caliper measurements, mice were randomized into two experimental cohorts and treatment initiated. Mice were either treated with vehicle control or 100 mg/kg experimental compound daily P.O. for 7-14 days. At harvest, tissues were snap-frozen.

Frozen xenograft samples were thawed and lysed in Pierce IP lysis buffer. Clarified lysates were subjected to immunoaffinity purification of MHC. Each sample was split in half and human pan class I was captured directly using W6/32 conjugated resin or following an initial depletion of mouse H-2d with M1/42-conjugated resin. Peptides (50%) were concentrated and desalted using solid-phase extraction (SPE) with Waters µHLB C18 plate. Peptides were loaded directly and eluted using 80/20 acetonitrile/water (0.1% TFA). Eluted peptides were lyophilized and reconstituted in 0.1% TFA.

Peptides (100%) were analyzed by nano LC/MS/MS using a Waters NanoAcquity system interfaced to a ThermoFisher Fusion Lumos mass spectrometer. Peptides were loaded on a trapping column and eluted over a 75 µm analytical column at 350 nL/min; both columns were packed with XSelect CSH C18 resin (Waters). A 2-hour gradient was employed. The mass spectrometer was operated using a custom data-dependent method, with MS performed in the Orbitrap at 60,000 FWHM resolution and sequential MS/MS performed using high resolution CID and EThcD in the Orbitrap at 15,000 FWHM resolution. All MS data were acquired from m/z 300-1600. A 3-second cycle time was employed for all steps.

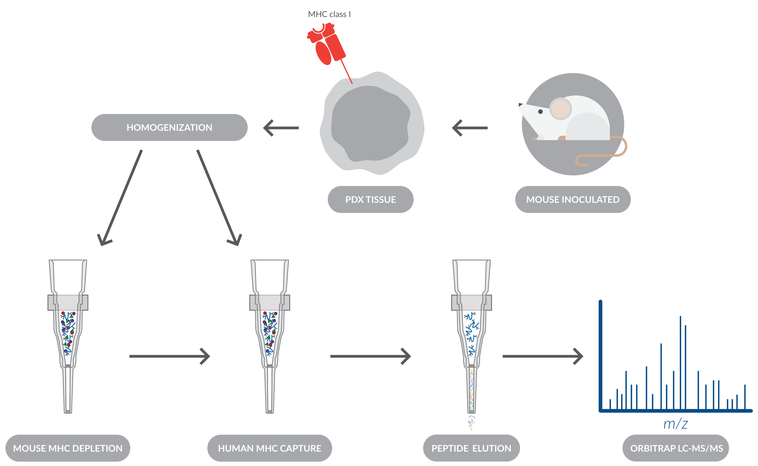

FIGURE 1 – Experimental workflow.

RESULTS

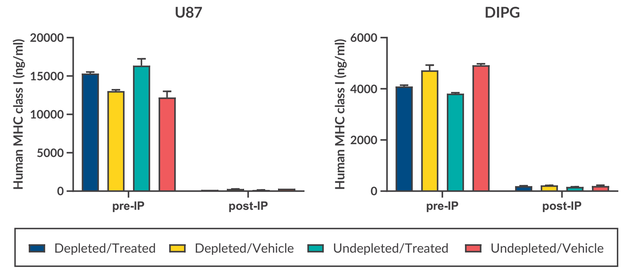

FIGURE 2 – Successful capture of MHC from PDX samples.

ELISA for human MHC class I (Item No. 502060, modified standard curve) was used to check capture of MHC complexes from homogenates of human glioma cells grown as tumors in nude mice. Similar MHC concentrations in depleted (subjected to previous immunoaffinity capture of mouse MHC class I) and undepleted samples indicates no non-specific capture of human class I by the mouse MHC antibody. Near complete loss of human MHC in post-IP (orange) samples suggests successful capture of MHC on the resin.

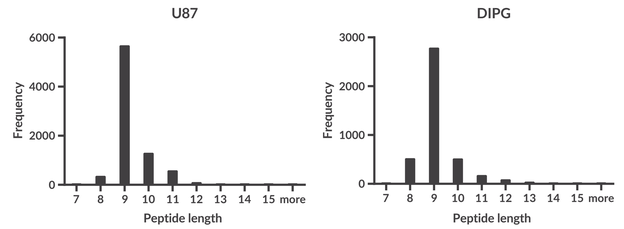

FIGURE 4 – Peptide lengths captured from MHC of PDX samples.

Peptides from both sample types show 8-11mer lengths typical of human MHC class I peptide lists.

ANALYSIS

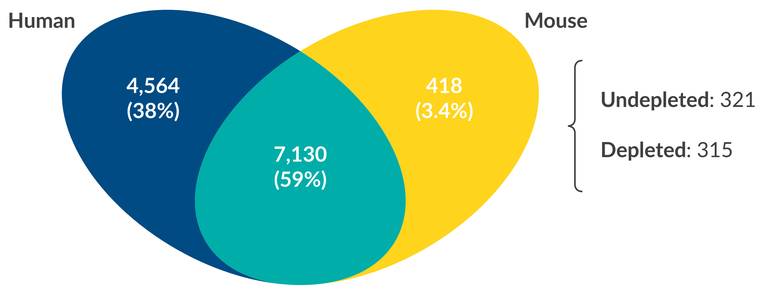

FIGURE 6 – Species origin of peptides identified in MHC of PDX samples.

MS data were searched using both mouse and human canonical proteomes. Using accession numbers, each peptide was assigned to a category: human only, mouse only, or both mouse and human (peptides which are identical in mouse and human proteomes). Only 418 peptides (3.4% of the total) were found to be specifically of mouse origin, despite the tumors being grown in a murine host. These are most likely attributable to uptake of host cells or proteins and cross-presentation by tumor cells in human class I complexes. Furthermore, there was no effect of mouse MHC depletion on the numbers of mouse peptides recovered, further suggesting that mouse MHC was not nonspecifically pulled down by the human-specific immunoaffinity resin.

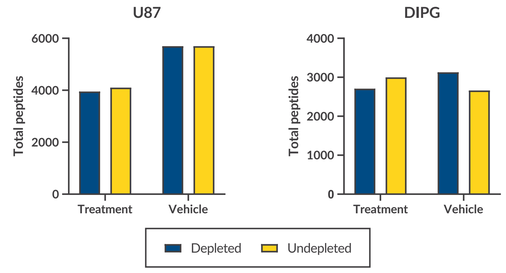

FIGURE 3 – Peptide totals from PDX immunopeptide profiling.

Eluted ligands from all samples were subjected to LC/MS-MS in a Fusion Lumos mass spectrometer. Data were searched against the canonical human proteome to obtain a complete list of human-derived peptides presented by the tumor cells in the mouse model. Similar numbers of peptides in depleted and undepleted samples suggests no loss of human peptides in the process of pre-depletion of mouse MHC.

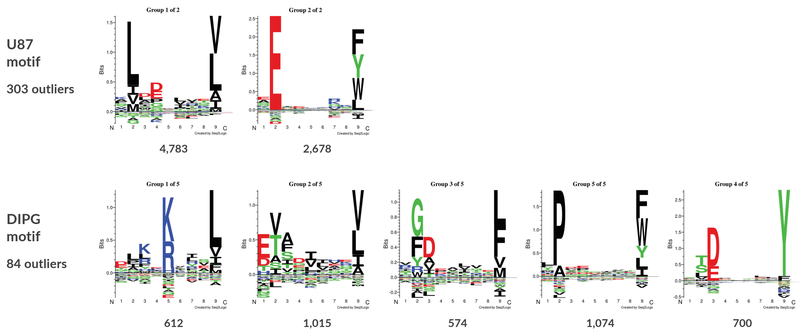

FIGURE 5 – Motif analysis of peptides from MHC of PDX samples.

Peptides from each sample type were clustered in an unbiased fashion using GibbsCluster and the optimal solution for each list is shown. The resulting motifs correspond to the indicated number of peptides, with the outlier numbers on the left.

CONCLUSIONS

- W6/32 resin successfully captures MHC class I from human cells grown in mouse PDX models.
- Pre-depletion of mouse MHC does not have a dramatic effect on the total number of peptides recovered from PDX tumor tissue.
- Peptides recovered conform to typical MHC class I structures.
- Mouse peptides do not contribute greatly to the immunopeptidome recovered from PDX samples, even in mice with intact antigen presenting cell systems.

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