BACKGROUND AND RATIONALE

- Immunotherapy studies in cancer seek to identify non-normal peptide sequences (neoantigens) derived from tumor-specific proteoforms, and also aberrantly expressed tumor-associated antigens (TAAs) from reference protein sequences, which are bound to the class I or II major histocompatibility complex (MHC) and activate the immune system.
- Identification of neoantigens relies on next-generation sequencing (NGS) to identify genomic or transcriptional alterations that may encode non-normal, candidate neoantigen peptides, coupled with algorithms that predict their binding to the MHC.
- Mass spectrometry (MS)-based immunopeptidomics enrich the MHC and utilize LC-tandem mass spectrometry (LC-MS/MS) analysis to detect and identify MHC-bound peptides, directly confirming predicted candidates with potential for immunotherapy, including those carrying post-translational modifications (PTMs).

NEOANTIGEN DISCOVERY AND VERIFICATION: REQUIREMENTS AND SOLUTIONS

- Multi-omic integration of neoantigen tools from NGS data with MS-based peptidomics analysis tools is a necessity.
- Sequence database searching of immunopeptidomic MS/MS data to efficiently identify MHC-bound peptides using “no enzyme” constraints, identifying PTMs, and quantifying peptide abundance using label-free quantification (LFQ).
- Ideally these tools would be contained in a single environment, accessible by bench researchers complete with training resources to empower their adoption.

A solution: Integrated multi-omic analysis within the Galaxy ecosystem

- The Galaxy ecosystem offers a platform to build multi-omic workflows integrating NGS analysis tools, MS/MS analysis tools, complete with access on publicly available instances, and training via the Galaxy Training Network.
- Building on the Galaxy for proteomics (Galaxy-P) software suite, we are developing workflows for multi-omic neoantigen discovery and verification using state-of-the-art tools, including NGS analysis tools, the FragPipe tool suite (fragpipe.nesvilab.org) and PepQuery (www.pepquery.org).

RESULTS: NEOANTIGEN PREDICTION FROM NGS DATA

- Developments guided by ongoing multi-omic study of neoantigens/TAAs from malignant peripheral nerve sheath tumors (MPNST) cells.

RESULTS: PEPTIDE NEOANTIGEN DISCOVERY AND VERIFICATION

- Databases searching of immunopeptidomic MS/MS data for rapid identification of MHC-bound peptides enriched from MPNST cells.

FUTURE PLANS

- Optimize and harden workflows for production-ready deployment.
- Publish optimized workflows and tools on accessible Galaxy gateways and in the Galaxy Tool Shed.
- Develop Galaxy Training Network tutorials for online, on-demand access.

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