Predicted MHC peptide binding promiscuity explains MHC class I 'hotspots' of antigen presentation defined by mass spectrometry eluted ligand data

Peptides that bind to and are presented by MHC class I and class II molecules collectively make up the immunopeptidome. In the context of vaccine development, an understanding of the immunopeptidome is essential, and much effort has been dedicated to its accurate and cost-effective identification. Current state-of-the-art methods mainly comprise in silico tools for predicting MHC binding, which is strongly correlated with peptide immunogenicity. However, only a small proportion of the peptides that bind to MHC molecules are, in fact, immunogenic, and substantial work has been dedicated to uncovering additional determinants of peptide immunogenicity. In this context, and in light of recent advancements in mass spectrometry (MS), the existence of immunological hotspots has been given new life, inciting the hypothesis that hotspots are associated with MHC class I peptide immunogenicity. We here introduce a precise terminology for defining these hotspots and carry out a systematic analysis of MS and in silico predicted hotspots. We find that hotspots defined from MS data are largely captured by peptide binding predictions, enabling their replication in silico. This leads us to conclude that hotspots, to a great degree, are simply a result of promiscuous HLA binding, which disproves the hypothesis that the identification of hotspots provides novel information in the context of immunogenic peptide prediction. Furthermore, our analyses demonstrate that the signal of ligand processing, although present in the MS data, has very low predictive power to discriminate between MS and in silico defined hotspots.