WITHDRAWN

The immunopeptidomic landscape of breast cancer.

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Advances in cancer immunotherapy have led to long-term remission in patients with melanoma and non-small cell lung carcinoma, but not every patient benefits equally from immunotherapy. Knowing the HLA-presented antigenic repertoire on breast cancer, enables the definition of safe, patient-individualized immunotherapies. We employed mass spectrometry-based immunopeptidomics to map the HLA-presented antigenic landscape of 31 primary breast cancer tissues, 23 tumor adjacent benign tissues, and 6 healthy mamma tissues and identified 42,376 HLA-I and 46,939 HLA-II ligands. The median HLA-I immunopeptidome yield per sample is 1,811 (range: 37 - 7567). The median purity of each sample, as defined by the ratio of assigned HLA binders and all identified peptides, was 93% (range 18% - 99%). Overall, a median of 1,285 (range 13 - 9363) HLA-II ligands were identified per sample. HLA ligands are characterized by a slightly overlapping class-specific length distribution that is mirrored in this dataset. The majority of HLA-I ligands are 9mers, while most HLA-II ligands are 15mers and 16mers. We set out to determine tumor-associated antigens by comparatively profiling tumor immunopeptidomes with their corresponding adjacent benign tissues and a carefully curated benign dataset of HLA-I and -II ligands, respectively. The benign sample cohort comprises the multi-tissue ligandome from the HLA Ligand Atlas without tests, supplemented with a set of 6 benign mamma samples. We found 11,622 tumor-associated HLA-I ligands and 17,460 HLA-II ligands that are presented only on breast cancer specimens, >80% of each being individual to a patient and its tumor. Overall, we identified 44 HLA-I ligands (frequency > 5/31) and 46 HLA-II ligands (frequency > 6/31) frequently and exclusively presented in breast cancer patients. These frequent tumor-associated antigens are shared by molecular subtypes and are promising candidates for downstream immunogenicity testing. This approach provides direct evidence for HLA presentation and tumor-association of potential T cell targets as a basis for downstream validation and the development of safe T-cell-based immunotherapies.

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