Claims

1. Method for the identification and label-free quantification of MHC ligand peptides on primary tissue samples, comprising the steps of
a) providing at least one diseased primary tissue sample and at least one sample of primary healthy tissue preferably corresponding to the diseased tissue,
b) isolating MHC ligand peptides from said sample(s),
c) performing an HPLC-MS analysis on said MHC ligand peptides,
d) extracting the precursor ion signal intensity (area) for each signal, as derived from step c),
e) identifying the sequences of said MHC ligand peptides by fragment spectra clustering and database search, in order to group areas between different runs and samples, and normalizing areas within replicate runs to compensate technical performance/sensitivity differences between replicate runs resulting in average quantities for each peptide per sample including error estimates,
f) Assigning of said sequences to MHC alleles in order to generate allele-specific sequence subgroups for a comparison between different samples, and normalizing between different samples using the allele-specific subgroups to account for different sample sizes or MHC expression levels: results is relative quantities comparable between samples,
g) performing a data quality control based on peptide reproducibility for every sample, verifying that the total number of identified sequences and the number of sequences with small variances in areas is as high as possible,
h) calculating presentation profiles and scores in order to determine a potential over-presentation of MHC ligand peptides,
i) controlling the quality of identifications and area estimates, for example by visual inspection of the peptide identification results and of the area reproducibilites, or statistical analysis,
j) comparing the values detected in said at least one diseased primary tissue sample with the value(s) obtained from said at least one primary healthy tissues, and
k) quantifying said MHC ligand peptides.

2. Method according to claim 1, wherein said MHC ligand peptide is selected from a tumor associated peptide (TAA) or disease associated peptide (DAA).

3. Method according to claim 1 or 2, further comprising a selection of over-represented and/or tumor-specific MHC ligand peptides.

4. Method according to any of claims 1 to 3, wherein said diseased sample is a tumor sample or a sample of infected tissue.

5. Method according to any of claims 1 to 4, wherein controlling quality is performed using an automate and/or by manual inspection.

6. Method according to any of claims 1 to 5, further comprising at least one step selected from the group of
in step d) aligning the retention time to assign corresponding signals within replicate runs without a knowledge about the sequences, and/or assigning the corresponding signals between different samples by retention time alignment without a knowledge about the sequences, in step h) implementing relative presentation data from more than one peptide per gene to calculate a gene-centered over-presentation score, and in step i) performing an quality control based on spiked peptides.

7. Method according to any of claims 1 to 6, wherein the method is performed *in vitro*.

8. Method according to any of claims 1 to 7, wherein the steps of said method are performed in the order as indicated.

9. Method according to any of claims 1 to 8, wherein said method consists of the steps as indicated.

10. Method according to any of the claims 1 to 9, further comprising the step of synthesizing said at least one MHC ligand peptide as identified and/or quantified by said method on a synthesizer or manually.

11. Method according to claim 10, further comprising the step of testing said MHC ligand peptide as synthesized peptide for its immunogenicity.

12. Method according to any of the claims 1 to 11, wherein said sample(s) is/are derived from one individual.

13. Method according to claim 12, further comprising generating a personalized MHC ligand profile, preferably a personalized disease-specific MHC ligand profile, based on said MHC ligand peptides as identified and/or quantified.

14. Method according to any of the claims 1 to 13, wherein said total number of identified sequences and the number of sequences with small variances in areas is higher than 5, preferably higher than 25, more preferably higher than 50, and most preferred higher than 100.

Claims

1. Method for the identification and label-free quantification of at least one MHC ligand peptide on one or more primary tissue samples, said method comprising

a) isolating at least one MHC ligand peptide from at least a first healthy and a second diseased tissue sample,

b) performing an HPLC-MS analysis on said MHC ligand peptides in order to generate a peptide signal therefor,

c) determining the precursor ion signal area for each of said MHC ligand peptide signals,

d) identifying the sequences for each of said MHC ligand peptides,

e) determining an average quantity of the MHC ligand peptide by grouping areas between different runs, and normalizing said areas within replicate runs, in order to create an average value for each sample,

f) determining relative quantities of the MHC ligand peptide that are comparable between samples, comprising

f1) assigning the MHC ligand peptide to an allele-specific sequence subgroup for a comparison between different samples by identifying at least one MHC allele to which the MHC ligand peptide is expected to bind, and

f2) normalizing between different samples using the allele-specific subgroups to account for different sample sizes and/or MHC expression levels; and

f3) performing a data quality control based on peptide reproducibility for each sample,

g) determining overpresentation of the MHC ligand peptide by calculating a presentation profile and/or a presentation score for the MHC ligand peptide of said diseased sample and said healthy sample based on said average and relative quantities of the MHC ligand peptide, and

h) label-free quantification of the MHC ligand peptide.

2. The method of claim 1, wherein said method allows handling of tissue samples of different amounts and MHC expression levels.

3. The method of claim 1 or 2, wherein said diseased sample is a tumor sample or a sample of infected tissue.

4. The method of any of claims 1 to 3, wherein the method is capable of being performed on a high-throughput basis.

5. The method of any of claims 1 to 4, wherein said method is capable of being performed incrementally by increasing an existing dataset from earlier tested samples with data of new samples.

6. The method of any of claims 1 to 5, wherein at least five label-free replicate LC-MS runs are performed for each sample.

7. The method of claim 6, wherein the areas are normalized within replicate runs by a method comprising:
(e1) calculating a mean presentation value for each peptide from presentation values of all replicate runs per sample; and
(e2) computing a normalization factor for each peptide and each replicate run using said mean presentation value; and
(e3) averaging over all peptides results in run-wise normalization factors; and
(e4) applying the run-wise normalization factors to all peptides of a particular replicate run.

8. The method of claim 1, wherein normalizing between different samples is performed by a method comprising:
(f2α) calculating a mean presentation value of each peptide for all samples of a defined preparation antibody;
(f2β) computing a normalization factor for each peptide and sample using said mean presentation value as calculated in step(f2α); and
(f2γ) averaging over all peptides results in sample-wise normalization factors, and
(f2δ) applying sample-wise normalization factors to all peptides of the particular sample.

9. The method of any of claims 1 to 8, wherein the presentation score is a p-value of a linear mixed model or a p-value of a t-test.

10. The method of claim 9, wherein the presentation score is adjusted for multiple testing using false discovery rate.

11. The method of any of claims 1 to 10, wherein the sequence is identified by generating a consensus spectrum from the HPLC-MS analysis and at least one previous HPLC-MS analysis.

12. The method of claim 11, further comprising performing fragment spectra clustering, wherein clustering is performed by a growing k-means clustering algorithm.