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(54) Titre : METHODE DE CLASSIFICATION D'ESPECES TUMORALES REPOSANT SUR UNE METHYLATION DE L'ADN
(54) Title: DNA-METHYLATION BASED METHOD FOR CLASSIFYING TUMOR SPECIES

(57) **Abrégé/Abstract:**

The present invention pertains to methods for classifying tumorous diseases based on their specific genomic DNA methylation profile. The invention provides a method that allows for a classification of a tumor sample obtained from a patient by analysing a multitude, preferably genome wide, collection of CpG positions by comparison to a classification rule derived from a set of methylation data acquired from pre-classified tumor species. The invention is in particular useful for classifying brain tumor samples since brain tumors are characterized by a large variety of distinct tumor species which have different prognostic values and require in the clinic a for each species developed treatment regime.



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(57) Abstract: The present invention pertains to methods for classifying tumorous diseases based on their specific genomic DNA methylation profile. The invention provides a method that allows for a classification of a tumor sample obtained from a patient by analysing a multitude, preferably genome wide, collection of CpG positions by comparison to a classification rule derived from a set of methylation data acquired from pre-classified tumor species. The invention is in particular useful for classifying brain tumor samples since brain tumors are characterized by a large variety of distinct tumor species which have different prognostic values and require in the clinic a for each species developed treatment regime.

CLAIMS

1. An *in-vitro* Method for the classification, stratification and/or diagnosis of a tumor species, the method comprising the steps of
 - (a) Providing a tumor-sample to be classified obtained from a tumor of a patient and, optionally, isolating genomic DNA therefrom,
 - (b) Determining DNA methylation status of a multitude of independent genomic CpG positions in the genome of said tumor-sample, and
 - (c) Classifying the tumor species of the tumor-sample based on the methylation levels as determined in (b) using a classification-rule,
wherein the classification-rule is obtained by random forest analysis of a training-data-set, the training-data-set comprising pre-determined methylation data derived from multitude of pre-classified tumor species, wherein said pre-determined methylation data comprises the methylation status of said CpG positions in the genome of each of said pre-classified tumor species.
2. The method according to claim 1, wherein the tumor is a brain tumor, and wherein the tumor species is a brain tumor species.
3. The method according to claim 1 or 2, wherein determining of DNA methylation levels is performed with a genomic array or chip comprising probes which are specific for the methylation of at least 1000 CpG positions, such as the HumanMethylation450k-chip (Illumina).
4. The method according to any of claims 1 to 3, wherein determining DNA methylation comprises a bisulfite treatment of the DNA.
5. The method according to any of claims 1 to 4, wherein training of the classification-rule comprises a preceding step of selecting CpG position which of all CpG positions used provide the most pure splitting rules, and using said selected CpG positions as a training-data-optimization-set to train the classification-rule.

6. The method according to any of claims 1 to 5, wherein training of the classification-rule comprises a step of down-sampling for each tumor species the number of bootstrap samples to the minority class, the minority class being the lowest sample size of a tumor species in the training-data-set.
7. The method according to any of claims 1 to 6, comprising the further step (d) including the methylation data of the tumor sample as classified in (c) into the training-data-set to obtain an enhanced-training-data-set, and computing an enhanced-classification-rule by random forest analysis based on the enhanced-training-data-set.
8. The method according to any one of claims 1 to 7, wherein the pre-determined methylation data includes for each pre-classified tumor species the methylation status at said CpG position of at least two or more independent samples.
9. An *in-vitro* method for stratifying the treatment of a tumor patient, comprising the classification of the tumor species of the tumor of the patient according to a method of any of claims 1 to 8, and stratifying the treatment of the patient in accordance with the diagnosed tumor species.
10. A computer-implemented method for generating a classification-rule for aiding the classification of tumor samples in cancer diagnosis, the method comprising providing DNA methylation data of a multitude of independent genomic CpG positions of genomes of a multitude of diverse pre-classified tumor species of the same tumor type; computing a random forest of binary decision trees from the DNA methylation data, wherein in each binary decision tree of said random forest each node is a CpG position, and each terminal leave a specific tumor species, and each binary splitting rule is a methylation status at said CpG position.
11. The computer-implemented method according to claim 10, wherein the diverse tumor species are selected from metastatic tumors, tumors stemming from specific tissues, tumors in a specific stage, recurrent tumors, tumors having a specific genetic mutation, tumors of patients having different gender, age or genetic background.
12. The computer-implemented method according to claim 10 or 11, comprising a further preceding step of selecting a set of CpG positions out of all CpG positions in the methylation data, wherein the set of CpG positions comprise CpG positions which

provide the most pure splitting rules in the random forest analysis, and using the methylation data corresponding to said set of CpG positions as a training-data-optimization-set to train the classification-rule.

13. The computer-implemented method according to any of claim 10 to 12, wherein the methylation data includes for each pre-classified tumor species the methylation status at said CpG position of at least one, two, three, four, five, six or more independent samples.
14. The computer-implemented method according to any of claim 10 to 13, comprising a step of down-sampling for each tumor species the number of bootstrap samples to the minority class, the minority class being the lowest sample size of a tumor species in the methylation data.
15. A Computer-readable storage medium having computer-executable instructions stored, that, when executed, cause a computer to perform a method according to any of claims 10 to 14.