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(54) Titre : UTILISATION DE LRP5 EN TANT QUE MARQUEUR EPIGENETIQUE POUR L'IDENTIFICATION DE CELLULES IMMUNITAIRES, EN PARTICULIER LES LYMPHOCYTES B

(54) Title: LRP5 AS EPIGENETIC MARKER FOR THE IDENTIFICATION OF IMMUNE CELLS, IN PARTICULAR B-CELLS

(57) Abrégé/Abstract:
The present invention relates to a method, in particular an in vitro method, for identifying B cells, comprising analyzing the methylation status of at least one CpG position in the mammalian gene region for Low density lipoprotein receptor-related protein 5 (LRP5), wherein a demethylation or lack of methylation of said gene region is indicative for a B cell, when compared to a non-B cell. The analyses according to the invention can identify B cells on an epi-genetic level and distinguish them from all other cells in complex samples, such as, for example, other blood or immune cells. The present invention furthermore provides an improved method for quantifying B cells, in particular in complex samples. The method can be performed without a step of purifying and/or enriching cells, preferably in whole blood and/or non-trypsinized tissue. Further claimed are kits and specific primers and probes for identifying methylation.

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(54) **Title**: LRP5 AS EPIGENETIC MARKER FOR THE IDENTIFICATION OF IMMUNE CELLS, IN PARTICULAR B-CELLS

(57) **Abstract**: The present invention relates to a method, in particular an *in vitro* method, for identifying B cells, comprising analyzing the methylation status of at least one CpG position in the mammalian gene region for Low density lipoprotein receptor-related protein 5 (LRP5), wherein a demethylation or lack of methylation of said gene region is indicative for a B cell, when compared to a non-B cell. The analyses according to the invention can identify B cells on an epi-genetic level and distinguish them from all other cells in complex samples, such as, for example, other blood or immune cells. The present invention furthermore provides an improved method for quantifying B cells, in particular in complex samples. The method can be performed without a step of purifying and/or enriching cells, preferably in whole blood and/or non-trypsinized tissue. Further claimed are kits and specific primers and probes for identifying methylation.

# CLAIMS

1. A method for identifying B cells in a sample, comprising analyzing the methylation status of at least one CpG position in the mammalian gene region for low density lipoprotein receptor-related protein 5 (LRP5), wherein preferably said gene region as analyzed is positioned according to SEQ ID No. 1, wherein a demethylation of said gene region is indicative for a B cell, when compared to a non-B cell.

2. The method according to claim 1, wherein said at least one CpG position is present in the 5' region upstream from the transcription start, promoter region, the 5' or 3' untranslated regions, exon, intron, exon/intron border and/or in the 3' region downstream of the transcriptional stop of said gene region as analyzed.

3. The method according to claim 1 or 2, wherein said at least one CpG position is selected from a CpG selected from the CpG positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, and 24 in the amplicon according to SEQ ID No. 1, and is preferably selected from CpG positions 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, and 17 in a fragment of the amplicon No. 2249 according to the bisulfite-converted sequence according to SEQ ID No. 2 or 3.

4. The method according to any one of claims 1 to 3, wherein said analysis of the bisulfite convertibility comprises a method selected from a methylation specific enzymatic digest, bisulfite sequencing, an analysis selected from promoter methylation, CpG island methylation, MSP, HeavyMethyl, MethyLight, Ms-SNuPE, and other methods relying on a detection of amplified DNA.

5. The method according to any one of claims 1 to 4, further comprising a quantification of the relative amount of B cells based on comparing relative amounts of said methylation frequency in the region as analyzed with relative amounts of the methylation frequency in a control gene, such as, for example, GAPDH.

6. The method according to any one of claims 1 to 5, wherein said sample is selected from a mammalian body fluid, including human blood samples, or a tissue, organ or cell type blood sample, a sample of blood lymphocytes or a fraction thereof.

7. The method according to any one of claims 1 to 6, further comprising a distinguishing of said B cells from all or at least one of the cell types selected from follicular helper T cells, cytotoxic T-cells, granulocytes, monocytes, NK-cells, and T-helper cells.

8. The method according to any one of claims 1 to 7, wherein said method is performed without a step of purifying and/or enriching said cells to be identified, preferably using whole blood and/or non-trypsinized tissue.

9. The method according to any one of claims 1 to 8, further comprising the step of concluding on the immune status of said mammal based on said B cells as identified.

10. A method for monitoring the level of B cells in a mammal, comprising performing the method according to any one of claims 5 to 9, and furthermore comparing said relative amount of said cells as identified to a sample taken earlier or in parallel from the same mammal, and/or to a control sample.

11. The method according to any one of claims 1 to 10, further comprising measuring and/or monitoring the amount of said B cells in response to chemical and/or biological substances that are provided to said mammal.

12. The method according to any one of claims 1 to 11, wherein said mammal suffers from or is likely to suffer from autoimmune diseases, transplant rejections, infection diseases, cancer, and/or allergy.

13. A kit for identifying, quantifying, and/or monitoring B cells in a mammal based on the analysis of the bisulfite accessibility of CpG positions in the gene region of LRP5, comprising components for performing a method according to any of claims 1 to 12, in particular a kit comprising a) a bisulfite reagent, and b) materials for the analysis of the methylation status of CpG positions selected from the CpG positions in the region according to SEQ ID NO: 1, such as an oligomer selected from the sequences according to SEQ ID NOs: 4 to 11.

14. An oligomer according to any of SEQ ID No. 4 to 11, or the amplicon according to SEQ ID No. 1, 2 or 3.

15. Use of the kit according to claim 13, or of the oligomer or amplicon according to claim 14 for identifying, quantifying, and/or monitoring B cells in a mammal.