METHOD OF DETERMINING THE RISK OF DEVELOPING BREAST CANCER BY DETECTING THE EXPRESSION LEVELS OF microRNAs (miRNAs)

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of SG provisional application No. 1201501781W, filed 9 March 2015, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the field of molecular biology. In particular, the present invention relates to the use of biomarkers for the detection and diagnosis of cancer.

BACKGROUND OF THE INVENTION

[0003] Breast cancer is the most common cancer afflicting women globally, despite improvements in cancer screening. Currently, the most widely used method for breast cancer screening is mammography, with sensitivity varying from 71% to 96% and specificity in the range of 94% to 97% but with a lower sensitivity in younger women. False-positive mammograms are common occurrences in breast cancer screening programs, which result in unnecessary additional breast imaging and biopsies, and cause psychological distress to many women. The diagnosis of breast cancer relies mainly on the histological examination of tissue biopsies, or cytology of fine-needle aspirates (FNA). An attractive alternative is the use blood-based tests. To date, serum tumour markers such as CA15.3 or BR27.29 have low sensitivity and thus are not used for breast cancer detection. There is thus a need for minimally invasive methods to improve detection and early diagnosis of breast cancer.

SUMMARY

[0004] In one aspect, the present invention refers to a method of determining the risk of developing breast cancer in a subject or determining whether a subject suffers from breast cancer, the method comprising detecting the expression level of hsa-miR-186-5p (SEQ ID NO: 77) and/or hsa-miR-409-3p (SEQ ID NO: 178) in a bodily fluid sample obtained from the subject and determining whether it is upregulated or downregulated as compared to a control, wherein upregulation of hsa-miR-186-5p (SEQ ID NO: 77) and/or downregulation of hsa-miR-409-3p (SEQ ID NO: 178) indicates that the subject has breast cancer or is at a risk of developing breast cancer. In another aspect, the present invention refers to a method of determining the risk of developing breast cancer in a subject or determining whether a subject suffers from breast cancer, the method comprising detecting the expression level of at least 5 miRNA listed in Table 8 in a bodily fluid sample obtained from the subject and determining whether the miRNA are upregulated or downregulated as compared to a control, wherein downregulation of hsa-miR-409-3p (SEQ ID NO: 178) indicates that the subject has breast cancer or is at a risk of developing breast cancer.

[0005] In one aspect, the present invention refers to a method of determining the risk of developing breast cancer in a subject or determining whether a subject suffers from breast cancer, comprising the steps of detecting the presence of miRNA in a bodily fluid sample obtained from the subject; measuring the expression level of at least 5 miRNA listed in Table 8 in the bodily fluid sample; and using a score based on the expression level of the miRNAs measured previously to predict the likelihood of the subject to develop or to have breast cancer, wherein the hsa-miR-409-3p (SEQ ID NO: 178), hsa-miR-382-5p (SEQ ID NO: 177), hsa-miR-375 (SEQ ID NO: 173), or hsa-miR-23a-3p (SEQ ID NO: 112) is downregulated in the subject, as compared to a control. In another aspect, the present invention refers to a method of determining the risk of developing breast cancer in a subject or determining whether a subject suffers from breast cancer, comprising the steps of detecting the presence of miRNA in a bodily fluid sample obtained from the subject; measuring the expression level of at least two miRNA listed in Table 14 in the bodily fluid sample; and using a score based on the expression level of the miRNAs measured previously to predict the likelihood of the subject to develop or to have breast cancer, wherein one of the miRNA listed in

CLAIMS

1. A method of determining the risk of developing breast cancer in a subject or determining whether a subject suffers from breast cancer, the method comprising detecting the expression level of at least 5 miRNA listed in Table 8 in a bodily fluid sample obtained from the subject and determining whether the miRNA are upregulated or downregulated as compared to a control, wherein downregulation of hsa-miR-409-3p (SEQ ID NO: 178) indicates that the subject has breast cancer or is at a risk of developing breast cancer.

2. The method of claim 1, wherein the method further comprises measuring the expression level of at least one further microRNA (miRNA) as listed in any one of Table 9, Table 10, Table 11, Table 12, or Table 13, wherein the further microRNA (miRNA) is different from the miRNA selected according to claim 1, and wherein the up- or downregulation is as indicated in the respective table; optionally,
wherein the method measures the differential expression level of at least one further miRNA as listed in Table 12 or 13, optionally,
wherein differential expression of miRNA expression in the sample obtained from the subject, as compared to the control, is indicative of the subject having breast cancer; optionally,
wherein upregulation of miRNAs as listed as "upregulated" in Table 12, as compared to the control, diagnoses the subject to have breast cancer; optionally,
wherein downregulation of miRNAs as listed as "downregulated" in Table 12 as compared to the control, diagnoses the subject to have breast cancer.

3. The method of claim 2, wherein differential expression of miRNA expression in the sample obtained from the subject, as compared to a control, is indicative of the subject having any one of the breast cancer subtypes selected from the group consisting of luminal A breast cancer subtype, Her2 overexpression (HER) breast cancer subtype and triple negative (TN or basal) breast cancer subtype.

4. The method of claim 3, wherein upregulation of miRNAs as listed as “upregulated” in Table 9, as compared to the control, diagnoses the subject to have luminal A breast cancer subtype; optionally, wherein downregulation of miRNAs as listed as "downregulated" in Table 9 as compared to the control, diagnoses the subject to have luminal A breast cancer subtype.

5. The method of claim 3, wherein upregulation of miRNAs as listed as “upregulated” in Table 10, as compared to the control, diagnoses the subject to have HER breast cancer subtype; optionally,

wherein downregulation of miRNAs as listed as "downregulated" in Table 10 as compared to the control, diagnoses the subject to have HER breast cancer subtype.

6. The method of claim 3, wherein upregulation of miRNAs as listed as “upregulated” in Table 11, as compared to the control, diagnoses the subject to have triple negative (TN) breast cancer subtype; optionally, wherein downregulation of miRNAs as listed as "downregulated" in Table 11 as compared to the control, diagnoses the subject to have triple negative (TN) breast cancer subtype.

7. The method of any one of the preceding claims, wherein the control is a sample obtained from a breast cancer-free subject.

8. A method of determining the risk of developing breast cancer in a subject or determining whether a subject suffers from breast cancer, comprising the steps of:
- detecting the presence of miRNA in a bodily fluid sample obtained from the subject;
- measuring the expression level of at least 5 miRNA listed in Table 8 in the bodily fluid sample; and
- using a score based on the expression level of the miRNAs measured previously to predict the likelihood of the subject to develop or to have breast cancer,
wherein the hsa-miR-409-3p (SEQ ID NO: 178), hsa-miR-382-5p (SEQ ID NO: 177), hsa-miR-375 (SEQ ID NO: 173), or hsa-miR-23a-3p (SEQ ID NO: 112) is downregulated in the subject, as compared to a control.

9. The method of 1 or claim 8, wherein the expression level of all of the miRNA listed in Table 14, is measured.

10. The method of claim 8, wherein the control for comparing the expression level of the at least 5 miRNA listed in Table 8 is a breast cancer-free subject.

11. The method of claim 8, wherein the method further comprises measuring the expression level of at least one further miRNAs, which when compared to a control, the expression level is not altered in the subject.

12. The method of claim 8 to 11, wherein the score is calculated using a classification algorithm selected from the group consisting of support vector machine algorithm, logistic regression algorithm, multinomial logistic regression algorithm, Fisher's linear discriminant algorithm, quadratic classifier algorithm, perceptron algorithm, k-nearest neighbours algorithm, artificial

neural network algorithm, random forests algorithm, decision tree algorithm, naive Bayes algorithm, adaptive Bayes network algorithm, and ensemble learning method combining multiple learning algorithms.

13. The method of claim 12, wherein the classification algorithm is pre- trained using the expression level of the control; optionally, wherein the classification algorithm compares the expression level of the subject with that of the control and returns a mathematical score that identifies the likelihood of the subject to belong to either one of the control groups; optionally, wherein the control is at least one selected from the group consisting of a breast cancer free control and a breast cancer patient.

14. The method of any one of claims 8 to 13, wherein the expression level of the miRNAs is any one of concentration, log(concentration), Ct/Cq number, two to the power of Ct/Cq number.

15. The method of any one of the preceding claims, wherein the breast cancer is an early stage breast cancer, and/or wherein the bodily fluid is selected from the group consisting of cellular and non-cellular components of amniotic fluid, breast milk, bronchial lavage, cerebrospinal fluid, colostrum, interstitial fluid, peritoneal fluids, pleural fluid, saliva, seminal fluid, urine, tears, whole blood, plasma, serum plasma, red blood cells, white blood cells and serum.