A problem with analyzing cell-free RNA in non-maternal and maternal blood is the lack of suitable data to estimate the biological causes of the cell-free RNA present. For example, there lacks a reliable method for determining tissue origins of the cell-free RNA present in blood.

Summary

In one aspect, the present invention refers to an *in vitro* method of assessing the health of a tissue, the method comprising: providing a biological sample of a subject, wherein the biological sample is a fluid selected from the group consisting of blood, a blood fraction, saliva, sputum, urine, semen, a transvaginal fluid, a cerebrospinal fluid, sweat, or a breast fluid; quantifying a sample level of RNA in the biological sample, wherein the RNA is cell-free RNA specific to a tissue of the subject; comparing the sample level of RNA to a reference level of the RNA and determining a health state of the tissue based on the comparing. In another aspect, the present invention refers to an *in vitro* method of assessing health of a tissue of a subject, the method comprising characterizing the tissue as abnormal if a specified level of RNA is present in the blood of the subject, wherein the RNA is cell-free RNA specific to the tissue of the subject. In one ~~aspect~~example, the present ~~invention refers to~~disclosure describes an in vitro method of assessing the health of a tissue, the method comprising detecting a sample level of RNA in a biological sample; comparing the sample level of RNA to a reference level of RNA specific to the tissue; determining whether a difference exists between the sample level and the reference level; and characterizing the tissue as abnormal if a difference is detected. In another ~~aspect~~example, the present ~~invention refers to~~ disclosure describes an in vitro method of assessing health of a tissue, the method comprising characterizing the tissue as abnormal if a specified level of RNA is present in the blood. In yet another ~~aspect~~example, the present ~~invention refers to~~ disclosure describes an in vitro method for detecting differential transcript levels from a biological sample comprising a mixture of genetic material from different genomic sources, the method comprising the steps of: a) obtaining a plurality of biological samples over different time-points, each sample containing a mixture of genetic material from different genomic sources; b) amplifying a plurality of RNA transcripts from the plurality of biological samples to obtain a plurality of amplified samples, each containing a mixture of amplified RNA transcripts from different genomic sources; c) detecting the levels of one or more of the RNA transcripts from each of the amplified samples; and d) conducting an analysis that compares the levels of one or more of the RNA transcripts between each of the amplified samples to determine a differential profile for one or more of the detected RNA transcripts across the different time-points.

Described herein are methods for profiling the origin of the cell-free RNA to assess the health of an organ or tissue. Deviations in normal cell-free transcriptomes are caused when organ/tissue-specific transcripts are released in to the blood in large amounts as those organs/tissue begin to fail or are attacked by the immune system or pathogens. As a result inflammation process can occur as part of body's complex biological response to these harmful stimuli. The disclosure, according to certain examples, utilizes tissue-specific RNA transcripts of healthy individuals to deduce the relative optimal contributions of different tissues in the normal cell-free transcriptome, with each tissue-specific RNA transcript of the sample being indicative of the apotopic rate of that tissue. The normal cell-free transcriptome serves as a baseline or reference level to assess tissue health of other individuals. The disclosure includes a comparative measurement of the cell-free transcriptome of a sample to the normal cell free
transcriptome to assess the sample levels of tissue-specific transcripts circulating in plasma and to assess the health of tissues contributing to the cell-free transcriptome.

In addition to normal reference levels, methods of the disclosure also utilize reference levels for cell-free transcriptomes specific to other patient populations. Using methods of the disclosure one can determine the relative contribution of tissue-specific transcripts to the cell-free transcriptome of maternal subjects, fetus subjects, and/or subjects having a condition or disease.

By analyzing the health of tissue based on tissue-specific transcripts, methods of the invention advantageously allow one to assess the health of a tissue without relying on disease-related protein biomarkers. In certain examples, methods of the disclosure assess the health of a tissue by comparing a sample level of RNA in a biological sample to a reference level of RNA specific to a tissue, determining whether a difference exists between the sample level and the reference level, and characterizing the tissue as abnormal if a difference is detected. For example, if a patient's RNA expression levels for a specific tissue differs from the RNA expression levels for the specific tissue in the normal cell-free transcriptome, this indicates that patient's tissue is not functioning properly.

What is claimed is:

1. An *in vitro* method of assessing the health of a tissue, the method comprising: providing a biological sample of a subject, wherein the biological sample is a fluid selected from the group consisting of blood, a blood fraction, saliva, sputum, urine, semen, a transvaginal fluid, a cerebrospinal fluid, sweat, or a breast fluid;

quantifying a sample level of RNA in the biological sample, wherein the RNA is cell-free RNA specific to a tissue of the subject;

comparing the sample level of RNA to a reference level of the RNA and

determining a health state of the tissue based on the comparing.

2. The method of claim 1, further comprising the step of monitoring the tissue for disease progression.

3. The method of claim 1, wherein the reference level corresponds to the state of the tissue at a time-point.

4. The method of claim 1, wherein the reference level is a level of RNA specific to the tissue in a healthy state.

5. The method of claim 1, wherein the tissue is not placental tissue or fetal tissue.

6. The method of claim 5, wherein the biological sample is blood.

7. The method of claim 1, wherein the biological sample is blood from which cells have been removed.

8. The method of claim 1, wherein the quantifying step is performed via a sequencing technique, a microarray technique, or both.

9. The method of claim 8, wherein the sequencing technique is whole transcriptome shotgun sequencing.

10. The method of claim 1, wherein said reference level is determined by a computer-generated database.

11. The method of claim 1, wherein the tissue is selected from the group consisting of: whole blood, bone marrow, hypothalamus, smooth muscle, lung, thymus, lymph node, heart, kidney, brain, cerebellum, liver, skin, and thyroid.

12. An *in vitro* method of assessing health of a tissue of a subject, the method comprising characterizing the tissue as abnormal if a specified level of RNA is present in the blood of the subject, wherein the RNA is cell-free RNA specific to the tissue of the subject.

13. The method of claim 12, wherein the tissue is selected from the group consisting of: whole blood, bone marrow, hypothalamus, smooth muscle, lung, thymus, lymph node, heart, kidney, brain, cerebellum, liver, skin, and thyroid.

14. The method of claim 12, further comprising

quantifying a sample level of the cell-free RNA in a blood sample;

comparing the sample level of the cell-free RNA to a reference level of the cell-free RNA specific to the tissue; and

determining whether a difference exists between the sample level and the reference level.

15. The method of claim 14, further comprising characterizing the tissue as abnormal if the sample level and the reference level are the same, wherein the reference level is indicative of a disease or a condition.

16. The method of claim 14, further comprising characterizing the tissue as abnormal if the sample level and the reference level are different, wherein the reference level is indicative of a healthy state.

17. The method of claim 14, wherein the quantifying step is performed via a sequencing technique, a microarray technique, or both.

18. The method of claim 17, wherein the sequencing technique is whole transcriptome shotgun sequencing.

19. The method of claim 14, wherein said reference level is determined by a computer-generated database.

20. The method of claim 1 or 12, wherein the cell-free RNA is a transcript of a gene selected from Figure 18.

21. The method of claim 1 or 14, wherein the sample level comprises cell-free RNA levels for a plurality of genes, and the reference level comprises cell-free RNA levels for each of the plurality of genes.

22. The method of claim 21, wherein the tissue is a brain tissue, and the plurality of genes are selected from the group consisting of: PMCH, PMCHL1, ABHD8, BAD, C12orf43, CAMK1G, CDK5R2, CHST10, CLCN6, FAM108B1, FAM50B, FBXL15, GPR17, GSTT2, ITPKA, KIF5A, KLHL26, NELL1, PIAS4, PPP2R2D, RAB33A, RAC3, RAPGEFL1, THAP10, and TIMM22.

23. The method of claim 1 or 14, wherein the reference level is a level indicative of a neurological disorder.

24. The method of claim 23, wherein the neurological disorder is multiple sclerosis, Alzheimer's disease, Huntington's disease, or amyotrophic lateral sclerosis.

What is claimed is:

1. An *in vitro* method of assessing the health of a tissue, the method comprising

detecting a sample level of RNA in a biological sample;

comparing the sample level of RNA to a reference level of RNA specific to the tissue;

determining whether a difference exists between the sample level and the reference level; and

characterizing the tissue as abnormal if a difference is detected.

2. The method of claim 1, further comprising the step of monitoring the tissue for disease progression.

3. The method of claim 1, wherein the reference level corresponds to the state of the tissue at a time-point.

4. The method of claim 1, wherein the reference level is a level of RNA specific to the tissue in a healthy state.

5. The method of claim 1, wherein the biological sample is blood, a blood fraction, saliva, sputum, urine, semen, transvaginal fluid, cerebrospinal fluid, stool, a cell or a tissue biopsy.

6. The method of claim 5, wherein the biological sample is blood.

7. The method of claim 6, wherein the RNA is cell-free RNA.

8. The method of claim 1, wherein the detecting step is performed via a sequencing technique, a microarray technique, or both.

9. The method of claim 8, wherein the sequencing technique is whole transcriptome shotgun sequencing.

10. The method of claim 1, wherein said reference level is determined by a computer-generated database.

11. The method of claim 1, wherein the tissue is selected from the group consisting of: whole blood, bone marrow, hypothalamus, smooth muscle, lung, thymus, lymph node, and thyroid.

12. An *in vitro* method of assessing health of a tissue, the method comprising characterizing the tissue as abnormal if a specified level of RNA is present in the blood.

13. The method of claim 12, wherein the tissue is selected from the group consisting of: whole blood, bone marrow, hypothalamus, smooth muscle, lung, thymus, lymph node, and thyroid.

14. The method of claim 12, further comprising

detecting a level of RNA in a blood sample;

comparing the sample level of RNA to a reference level of RNA specific to a tissue;

determining whether a difference exists between the sample level and the reference level, and

characterizing the tissue as abnormal if the sample level and the reference level are the same.

15. The method of claim 14, wherein the reference level is indicative of a disease or a condition.

16. The method of claim 12, wherein the RNA is cell-free RNA.

17. The method of claim 12, wherein the detecting step is performed via a sequencing technique, a microarray technique, or both.

18. The method of claim 17, wherein the sequencing technique is whole transcriptome shotgun sequencing.

19. The method of claim 14, wherein said reference level is determined by a computer-generated database.

20. An *in vitro* method for detecting differential transcript levels from a biological sample comprising a mixture of genetic material from different genomic sources, the method comprising the steps of:

a) obtaining a plurality of biological samples over different time-points, each sample containing a mixture of genetic material from different genomic sources;

b) amplifying a plurality of RNA transcripts from the plurality of biological samples to obtain a plurality of amplified samples, each containing a mixture of amplified RNA transcripts from different genomic sources;

c) detecting the levels of one or more of the RNA transcripts from each of the amplified samples; and

d) conducting an analysis that compares the levels of one or more of the RNA transcripts between each of the amplified samples to determine a differential profile for one or more of the detected RNA transcripts across the different time-points.

21. The method of claim 20, wherein the biological sample is blood, a blood fraction, saliva, sputum, urine, semen, transvaginal fluid, cerebrospinal fluid, stool, a cell or a tissue biopsy.

22. The method of claim 21, wherein the biological sample is blood.

23. The method of claim 22, wherein the blood is peripheral blood derived from a pregnant woman, or a fraction thereof.

24. The method of claim 20, wherein the different genomic sources are derived from a pregnant female and a fetus.

25. The method of claim 20, wherein the detecting step is performed via a sequencing technique, a microarray technique, or both.

26. The method of claim 25, wherein the sequencing technique is whole transcriptome shotgun sequencing.

27. The method of claim 25, wherein the differential profile of one or more RNA transcripts is indicative of a disease or a condition.

28. The method of claim 27, wherein the disease or condition is preterm pregnancy.

29. The method of claim 27, wherein the disease or condition is a pathological pregnancy.

30. The method of claim 27, wherein the differential profile includes one or more genes selected from the group consisting PVALB, CLCN3, ITGA2B, LTV1, HIST1H4B, TREML1, NPTN, LSM2, SCGB1C1, NOP10, MFSD1, MALAT1, GDI1, HIST1H1C, HIST1H4H, CD226, ITM2B, MLLT6, ANO6, and ITGB3.