METHODS AND SYSTEMS FOR DETECTING TISSUE CONDITIONS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application 62/560,936 filed on September 20, 2017 pursuant to 35 USC 119, which is incorporated by reference as if set forth fully herein.

BACKGROUND OF THE INVENTION

[0002] A variety of markers are available for detecting various conditions. However, many of these conditions are ones that can affect different tissues. Detecting markers of these conditions in circulation, such as in a blood sample, are not always helpful in identifying which tissue is affected. For example, generic markers for inflammation can indicate an inflammatory response somewhere in the body, but it may not be known which tissue is suffering the response, such as the liver, kidney, lungs, or joints. Tissue-specific tests, such as biopsies, are often invasive, carrying a risk of infection, and typically not comprehensive of the entire organ or tissue. Imaging techniques, such as MRIs and CT-scans, may be used to assess tissue health, but generally can only detect overt features and changes. Thus, these imaging techniques are generally not sensitive enough to pick up early onset of conditions or fairly recent developments of conditions.

SUMMARY OF THE DISCLOSURE

[0002a] In one aspect, provided herein is a method comprising:

a) removing cells from a blood sample obtained from a subject to obtain a cell-depleted sample;

b) removing an extracellular microparticle from the cell-depleted sample to obtain a microparticle-depleted sample;

c) quantifying an amount of cell-free mRNA corresponding to a gene in the microparticle-depleted sample

d) comparing the amount of cell-free mRNA corresponding to the gene to a reference level of the gene; and

e) determining a health state of a tissue of the subject based on the comparing.

[0003] Disclosed herein, in some examples, are methods comprising: obtaining a blood sample from a subject; removing cells from the blood sample to obtain a cell-depleted sample; removing an extracellular microparticle from the blood sample to obtain a microparticle-depleted sample; quantifying an amount of cell-free RNA corresponding to a gene in the microparticle-depleted sample. In some instances, removing the extracellular microparticle comprises contacting the cell-depleted sample with a binding moiety that interacts with a protein on the surface the extracellular microparticle. In some instances, the binding moiety is an antibody or antigen-binding antibody fragment. In some instances, the antibody or antigen-

CLAIMS

WHAT IS CLAIMED IS:

1. A method comprising:
   a) removing cells from a blood sample obtained from a subject to obtain a cell-depleted sample;
   b) removing an extracellular microparticle from the cell-depleted sample to obtain a microparticle-depleted sample;
   c) quantifying an amount of cell-free mRNA corresponding to a gene in the microparticle-depleted sample;
   d) comparing the amount of cell-free mRNA corresponding to the gene to a reference level of the gene; and
   e) determining a health state of a tissue of the subject based on the comparing.
2. The method of claim 1, wherein removing the extracellular microparticle further comprises contacting the cell-depleted sample with a binding moiety that interacts with a protein on a surface of the extracellular microparticle.
3. The method of claim 2, wherein the binding moiety is an antibody or an antigen-binding antibody fragment.
4. The method of claim 3, wherein the antibody or the antigen-binding antibody fragment interacts with a cell surface marker known to be expressed by a blood cell.
5. The method of claim 3, wherein the antibody comprises an anti-CD45 antibody.
6. The method of claim 3, wherein the antibody comprises an anti-CD66b antibody.
7. The method of claim 1, further comprising contacting the cell-depleted sample with a first antibody that interacts with a first protein on a first cell and a second antibody that interacts with a second protein on a second cell.
8. The method of claim 7, wherein the first protein is not expressed on the second cell, and wherein the second protein is not expressed on the first cell.
9. The method of claim 1, wherein the extracellular microparticle is an exosome.
10. The method of claim 9, wherein the exosome is from a blood cell.
11. The method of claim 10, wherein the blood cell is a platelet, and wherein removing the extracellular microparticle further comprises contacting the cell-depleted sample with an anti-GypA antibody or a GypA antigen-binding fragment thereof.
12. The method of claim 10, wherein the blood cell is a red blood cell, and wherein removing the extracellular microparticle further comprises contacting the cell-depleted

sample with an anti-CD235a antibody or a CD235a antigen-binding antibody fragment thereof.

13. The method of claim 10, wherein the blood cell is a granulocyte, and wherein removing the extracellular microparticle further comprises contacting the cell-depleted sample with an anti-CD66b antibody or a CD66b antigen-binding antibody fragment thereof.

14. The method of claim 10, wherein the blood cell is a lymphocyte, and wherein removing the extracellular microparticle further comprises contacting the cell-depleted sample with an antibody or an antigen-binding antibody fragment thereof that binds CD45, CD19 or CD3.

15. The method of claim 1, wherein (c) further comprises incubating the cell-depleted sample for 1 hour at 60 degrees Celsius.

16. The method of claim 15, further comprising incubating the cell-depleted sample in the presence of a chaotropic salt, a detergent, a proteinase K, a 2-mercaptoethanol, or a combination thereof.

17. The method of claim 16, further comprising incubating the cell-depleted sample with Tris.

18. The method of claim 1, further comprising disrupting lipidic and proteinaceous structures.

19. The method of claim 1, further comprising contacting the cell-depleted sample with a silica column.

20. The method of claim 19, further comprising incubating the cell-depleted sample with a chaotropic salt and 100% isopropanol to enhance binding of RNA to the silica column.

21. The method of claim 1, wherein (d) further comprises sequencing the cell-free mRNA.

22. The method of claim 1, wherein (d) further comprises performing quantitative polymerase chain reaction (qPCR) on the cell-free mRNA.

23. The method of claim 1, wherein (d) further comprises performing microarray analysis of the cell-free mRNA.

24. The method of claim 1, wherein the tissue comprises liver tissue, adipose tissue, kidney tissue, pancreas tissue, heart tissue, muscle tissue, bone marrow tissue, skin tissue, prostate tissue, testes tissue, breast tissue, ovary tissue, uterus tissue, colon tissue, lung tissue, or neurological tissue.

25. The method of claim 24, wherein the tissue comprises liver tissue.

26. The method of claim 24, wherein the tissue comprises adipose tissue.

27. The method of claim 24, wherein the tissue comprises kidney tissue.

28. The method of claim 24, wherein the tissue comprises neurological tissue.

29. The method of claim 28, wherein the neurological tissue comprises brain, spinal cord, peripheral nerves, or a combination thereof.

30. The method of claim 1, wherein the health state comprises a presence or an absence of a disease or disorder of the tissue of the subject.

31. The method of claim 30, wherein the disease or disorder comprises cancer, cardiovascular disease, hepatitis, metabolic syndrome, obesity, diabetes, or a neurodegenerative disorder.