SYSTEMS, COMPOSITIONS, AND METHODS FOR TARGET ENTITY DETECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims the benefit of U.S. Provisional Application No. 62/812,878 filed March 01, 2019, and U.S. Provisional Application No. 62/962,722 filed January 17, 2020, the contents of each of which are hereby incorporated herein in their entirety.

BACKGROUND

[2] Early detection of cancer greatly increases the chance of successful treatment. However, many cancers still lack effective screening recommendations. Typical challenges for cancer-screening tests include limited sensitivity and specificity. A high rate of false-positive results can be of particular concern, as it can create difficult management decisions for clinicians and patients who would not want to unnecessarily administer (or receive) anti-cancer therapy that may potentially have undesirable side effects. Conversely, a high rate of false-negative results fails to satisfy the purpose of the screening test, as patients who need therapy are missed, resulting in a treatment delay and consequently a reduced possibility of success.

SUMMARY

[2.1] The present disclosure, among other things, provides insights and technologies for achieving effective cancer screening. In one aspect, the present disclosure refers to a method comprising: contacting a sample with a first detection probe comprising a first target-binding moiety and a first oligonucleotide domain coupled to the first target-binding moiety, the first oligonucleotide domain comprising a first double-stranded portion and a first-single stranded overhang, and a second detection probe comprising a second target-binding moiety and a second oligonucleotide domain coupled to the second target-binding moiety, the second oligonucleotide domain comprising a second double-stranded portion and a second single-stranded overhang that is complementary to at least a portion of the first single-stranded overhang; wherein the first single-stranded overhang and the second single-stranded overhang hybridize together to form a double-stranded complex when the first and second target-binding moieties are bound to their respective targets; ligating at least one strand of the double-stranded complex to form a ligated template; and detecting the ligated template.

CLAIMS

What is claimed is:

1. A method comprising:

contacting a sample with a first detection probe comprising a first target-binding moiety and a first oligonucleotide domain coupled to the first target-binding moiety, the first oligonucleotide domain comprising a first double-stranded portion and a first-single stranded overhang, and a second detection probe comprising a second target-binding moiety and a second oligonucleotide domain coupled to the second target-binding moiety, the second oligonucleotide domain comprising a second double-stranded portion and a second single-stranded overhang that is complementary to at least a portion of the first single-stranded overhang; wherein the first single-stranded overhang and the second single-stranded overhang hybridize together to form a double-stranded complex when the first and second target-binding moieties are bound to their respective targets;

ligating at least one strand of the double-stranded complex to form a ligated template; and

detecting the ligated template.

2. The method of claim 1, wherein the detecting comprises performing amplification of the ligated template and detecting the presence of an amplification product, optionally wherein the amplification is or comprises quantitative polymerase chain reaction.

3. The method of claim 1 or 2, further comprising immobilizing extracellular vesicles from the sample on a solid substrate prior to contacting the sample, optionally wherein the solid substrate is or comprises a bead.

4. The method of any one of claims 1-3, wherein the first target-binding moiety and/or the second target-binding moiety comprise(s) an antibody agent.

5. The method of any one of claims 1-4, further comprising contacting the sample with an additional detection probe for a third target, the additional detection probe comprising a third target-binding moiety, a third double-stranded portion, and a third single-stranded overhang.

6. The method of any one of claims 1-5, further comprising contacting the sample with 2-20 detection probes each for a specific target, each of the detection probes comprising a target-binding moiety, a double-stranded portion, and a single-stranded overhang.

7. The method of any one of claims 1-6, further comprising contacting the sample with a control probe, wherein the control probe is characterized in that binding of the control probe to a control reference inhibits generation of a ligated template and/or inhibits amplification of a ligated template from a non-target.

8. The method of claim 1, wherein the sample comprises a population of extracellular vesicles, optionally wherein at least one of the extracellular vesicles expresses a target biomarker signature for a disease, disorder, or condition, for example, wherein the disease, disorder, or condition is cancer, optionally
wherein the cancer is selected from the group consisting of bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancer, endometrial cancer, esophageal cancer, gastrointestinal cancer, Hodgkin lymphoma, kidney cancer, liver cancer, lung cancer, multiple myeloma, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, sarcomas, skin cancer, and stomach cancer.

9. The method of claim 1, wherein the sample comprises a blood-derived sample from a subject.

10. The method of claim 9, wherein the subject has at least one or more of the following characteristics:
    a. (i) an asymptomatic subject who is susceptible to cancer;
    (ii) a subject with a family history of cancer;
    (iii) a subject determined to have one or more germline mutations in one or more cancer-associated genes;
    (iv) an elderly subject;

(v) a subject with one or more non-specific symptoms of cancer;

(vi) a subject recommended for periodic cancer screening;

(vii) a subject diagnosed with an imaging-confirmed mass;

(viii) a subject at hereditary risk for cancer before undergoing a risk-reducing surgical intervention;

(ix) a subject with a benign tumor; and

(x) a subject who has been previously treated for cancer; or

b. the sample has been subjected to size exclusion chromatography to isolate nanoparticles having a size range of interest that includes extracellular vesicles; or

c. the method is used in combination with one or more of the following diagnostic assays:

(i) the subject's annual physical examination;

(ii) a cancer screening test;

(iii) a genetic assay to screen blood plasma for genetic mutations in circulating tumor DNA and/or protein biomarkers linked to cancer;

(iv) an assay involving immunofluorescent staining to identify cell phenotype and marker expression, followed by amplification and analysis by next-generation sequencing; and

(v) germline and somatic mutation assays, or assays involving cell-free tumor DNA, liquid biopsy, serum protein and cell-free DNA, and/or circulating tumor cells.

11. The method of claim 8, wherein the target biomarker signature comprises

(i) a first target biomarker comprising an extracellular vesicle-associated membrane-bound polypeptide; and

(ii) a second target biomarker comprising a target biomarker selected from the group consisting of: surface protein biomarkers, intravesicular protein biomarkers, and intravesicular RNA biomarkers.

12. The method of claim 1, further comprising performing a capture assay prior to contacting the sample.

13. The method of claim 12, wherein the capture assay involves contacting the sample with a capture agent comprising a target-capture moiety that binds to an extracellular vesicle-associated membrane-bound polypeptide.