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, a first double-stranded portion and a first-single stranded overhang, and a second detection probe comprising a second target-binding moiety, 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.

[2.2] In another example, the present disclosure refers to a method of detecting cancer, the method comprising steps of: detecting on surfaces of intact extracellular vesicles from a human blood sample co-localization of at least two biomarkers whose combined expression level has been determined to be associated with cancer; comparing the detected co-localization level with the determined level; and detecting cancer when the detected co-localization level is at or above the determined level.

[2.3] In another example, the present disclosure refers to a method comprising steps of contacting a sample comprising exosomes with a set of detection probes that specifically bind to surface biomarkers on the exosomes to detect cancer-associated exosomes in the sample with a specificity within a range of 95% to 100% and sensitivity within a range of 30% to 100%.

[2.4] In another example, the present disclosure refers to a method comprising steps of: capturing exosomes from a biological sample with a capture agent that selectively interacts with a cancer-specific surface protein biomarker on the exosomes; and contacting the captured exosomes with at least one set of at least two detection probes that each selectively interacts with a surface protein biomarker on the exosomes; and detecting a product formed when the at least two detection probes of the set are in sufficiently close proximity, such detection indicating co-localization of the surface protein biomarkers.

[2.5] In another example, the present disclosure refers to a method comprising steps of: contacting a sample comprising exosomes with a set of probes that specifically bind to surface biomarkers on the exosomes to detect cancer-associated exosomes in the sample, wherein: (i) each probe in the set comprises a target binding moiety directed to a surface biomarker on the exosomes; and (ii) the set comprises at least one capture probe and at least two detection probes, wherein each detection probe further comprises a detection moiety.

[2.6] In another example, the present disclosure refers to a method comprising steps of: performing a proximity assay that detects a surface biomarker signature on exosomes from a human subject, the step of performing being performed a period of time after a performance of a prior assay to detect the surface biomarker signature on exosomes from the human subject; and comparing results of the performed assay with those of the prior assay.

[2.7] In another example, the present disclosure refers to a method comprising steps of: contacting exosomes with at least two detection probes, wherein each detection probe comprises (i) a binding moiety; and (ii) an oligonucleotide entity, wherein the binding moiety is the same and the oligonucleotide entities complement one another.

[2.8] In another example, the present disclosure refers to a complex comprising: (a) an exosome having at least one target biomarker on its surface; and (b) a first detection probe and a second detection probe each bound to the exosome, wherein each of the first detection probe and the second detection probe comprises: (i) a target binding moiety directed to a target biomarker expressed by the exosome; and (ii) an oligonucleotide domain coupled to the target binding moiety, the oligonucleotide domain comprising a double-stranded portion and a single-stranded overhang portion extended from one end of the oligonucleotide domain, wherein the single-stranded overhang portions of the first and second detection probes are hybridized to each other.

[2.9] In another example, the present disclosure refers to a composition comprising extracellular vesicles from a human blood sample bound to a set of at least two probes, each of which comprises a biomarker binding moiety and an oligonucleotide domain, wherein two or more bound probes are in proximity to one another so that their oligonucleotide domains hybridize to each other to form a ligatable hybrid.

[2.10] In another example, the present disclosure refers to a complex comprising: (a) an exosome comprising a cancer-associated target biomarker signature; and (b) at least a first detection probe and a second detection probe each bound to the exosome, wherein each of the detection probes comprise: (i) a target binding moiety directed; to the target biomarker signature; and (ii) an oligonucleotide domain coupled to the target binding moiety, the oligonucleotide domain comprising a double-stranded portion and a single-stranded overhang portion extended from one end of the oligonucleotide domain, wherein the single-stranded overhang portions of the detection probes are at least partially complementary.

[2.11] In yet another example, the present disclosure refers to a set of probes, each of which comprises: (a) a biomarker binding moiety that specifically binds to a surface biomarker on extracellular vesicles from cancer cells; and (b) an oligonucleotide domain, wherein the oligonucleotide domains of probes within the set are arranged and constructed so that, when the probes are bound to their target biomarkers, their oligonucleotide domains hybridize to one another to form a ligatable hybrid only when the target biomarkers are in proximity to one another.

CLAIMS

What is claimed is:

1. A method comprising:

contacting a sample with a first detection probe comprising a first target-binding moiety, a first double-stranded portion and a first-single stranded overhang, and a second detection probe comprising a second target-binding moiety, 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.