RNA MARKERS AND METHODS FOR IDENTIFYING COLON CELL PROLIFERATIVE DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/017,552, filed April 29, 2020, and U.S. Provisional Patent Application No. 63/024,875, filed May 14, 2020, each of which is incorporated by reference herein in its entirety.

BACKGROUND

[0002] Cancer screening and early detection are considered the most efficient strategies against cancer because detecting malignancy or a precursor lesion at an early stage prior to the onset of symptoms are when treatments are most effective. In colorectal cancer, for instance, colonoscopies play a role in improving early diagnosis. While colonoscopies are useful for early detection, patient compliance rates are low, and screening is conducted below recommended regularity due to the invasiveness of the procedure. Thus, non-invasive methods offer a more promising approach for early cancer detection.

SUMMARY

[0003] The present disclosure provides methods and systems directed to micro ribonucleic acid (microRNA, or miRNA) profiling of genes associated with colon cell proliferative disorder (e.g., colorectal cancer) detection and disease progression. Some examples of the present disclosure provide miRNAs that are differentially abundant in a sample of a subject having a colon cell proliferative disorder, or having a high risk of developing colon cell proliferative disorder, as compared to the corresponding sample of a subject not having colon cell proliferative disorder, or having low risk of developing colon cell proliferative disorder. In some examples, each of the subjects having high risk of developing colon cell proliferative disorder and the subjects having low risk of developing colon cell proliferative disorder have a non-invasive precursor lesion arising within colorectal mucosa (hereinafter, colorectal lesion). The miRNAs that are present at different abundances (often referred to as “differentially expressed”) in a sample of a subject having colon cell proliferative disorder, or having a high risk of developing colon cell proliferative disorder, can be used as biomarkers for diagnosis, treatment, and/or prevention of colon cell proliferative disorder.

[0004] The miRNAs identified herein can be used to identify subjects that have colon cell proliferative disorder to distinguish them from subjects that do not have colon cell proliferative disorder, or to identify subjects having a higher risk of developing colon cell proliferative

disorder to distinguish them from subjects that have a lower risk of developing colon cell proliferative disorder, or to identify subjects having a colon cell proliferative disorder precursor (such as intraductal papillary mucinous neoplasm (IPMN)) versus a non-IPMN, or to identify subjects that have a malignant IPMN versus a benign IPMN. Thus, these miRNAs can be used as an adjunctive tool to guide decisions regarding monitoring, treatment, and management of colon cell proliferative disorder.

[0005] Some examples of the present disclosure provide a machine learning model classifier trained on the miRNAs described herein that are differentially expressed in a sample of a subject having colon cell proliferative disorder, or having a high risk of developing colon cell proliferative disorder, for example, when the subject has a colorectal lesion. In an example, a method is provided for a blood-based minimally-invasive miRNA assay that can be used in a subject having a colorectal lesion to assess histologic severity. In another example, the miRNAs indicative of colon cell proliferative disorder are detected in cell-free samples from a subject, for example, bodily fluid samples from a subject, such as whole blood, plasma, or serum. As such, the present disclosure provides miRNAs that can be used to differentiate between the presence or absence of colon cell proliferative disorder, high-risk or low-risk colorectal lesions that warrant treatment such as, surgical resection, immunotherapy, radiation, or chemotherapy) and low-risk colorectal lesions that can be monitored. Monitoring and confirmation of the presence of colon cell proliferative disorder or lesions can be carried out, for example, by colonoscopy, ultrasound, MM, or CT scan.

[0006] In an aspect, the present disclosure provides a micro ribonucleic acid (miRNA) signature panel characteristic of a colon cell proliferative disorder, comprising: a pre-determined set of one or more, two or more, three or more, or four or more miRNAs selected from the group listed in **Tables 1-11**, wherein the four or more miRNAs are differentially expressed between a biological sample from a subject having the colon cell proliferative disorder or subtype thereof, and a biological sample from a subject without the colon cell proliferative disorder or subtype thereof.

[0007] In some examples, the miRNA signature panel is characteristic of advanced adenoma, and the signature panel comprises: a pre-determined set of miRNAs comprising: a) hsa-miR-1273a, hsa-miR-17-5p, hsa-miR-20a-3p, hsa-miR-20b-5p; b) hsa-miR-3065-5p, hsa-miR-4785, hsa-miR-5096, hsa-miR-5189-5p, or c) hsa-miR-545-3p, hsa-miR-570-3p, hsa-miR-624-3p, hsa-mir-1181, hsa-mir-6073, wherein the miRNAs are differentially expressed between a biological sample from a subject having advanced adenoma or subtype thereof, and a biological sample from a subject without advanced adenoma or subtype thereof.

[0008] In some examples, the miRNA signature panel is characteristic of colorectal cancer, and the signature panel comprises: a pre-determined set of miRNAs comprising: a) hsa-miR-1250-5p, hsa-miR-1255a, hsa-miR-223-3p, hsa-miR-338-3p, hsa-miR-338-5p; b) hsa-miR-424-5p, hsa-miR-424-3p, hsa-miR-450a-5p, hsa-miR-450b-5p, hsa-miR-4772-3p; c) hsa-miR-4772-5p, hsa-miR-625-5p, hsa-miR-7847-3p, hsa-miR-1181, hsa-miR-3651, hsa-mir-6073; d) hsa-mir-6125, hsa-mir-7704, hsa-miR-19b-3p, hsa-miR-19a-3p, hsa-miR-3157-5p; e) hsa-miR-142-3p, hsa-miR-30c-5p, hsa-miR-6741-5p, hsa-miR-590-3p, hsa-miR-4685-5p; f) hsa-miR-3648, hsa-miR-331-3p, hsa-miR-1303, hsa-miR-6790-3p, hsa-miR-6867-5p, hsa-miR-942-5p; g) hsa-miR-378a-3p, hsa-miR-1287-5p, hsa-mir-4785, hsa-miR-324-3p, hsa-miR-550b-2-5p; h) hsa-miR-200c-3p, hsa-miR-200b-3p, hsa-miR-3679-5p, hsa-miR-550a-3-5p, hsa-miR-3187-3p; i) hsa-miR-181b-5p, hsa-miR-3138, hsa-miR-146a-5p, hsa-miR-6721-5p, hsa-miR-23b-3p, hsa-miR-28-5p; j) hsa-miR-320d, hsa-miR-940, hsa-miR-320d-1, hsa-miR-10a-5p, hsa-miR-340-5p; k) hsa-miR-320b, hsa-miR-335-5p, hsa-miR-320c, hsa-miR-501-3p, hsa-miR-548n; or l) hsa-miR-27a-3p, hsa-miR-3065-3p, hsa-miR-548aa@, hsa-miR-584-3p, hsa-miR-22-3p, wherein the miRNAs are differentially expressed between a biological sample from a subject having the colorectal cancer or subtype thereof, and a biological sample from a subject without the colorectal cancer or subtype thereof.

[0009] In some examples, the pre-determined set of miRNAs comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, or at least 250 miRNAs selected from the group listed in **Tables 1-11**.

[0010] In some examples, the biological sample is selected from the group consisting of bodily fluid, stool, colonic effluent, urine, blood plasma, blood serum, whole blood, isolated blood cells, cells isolated from the blood, and combinations thereof.

[0011] In some examples, the biological sample comprises a nucleic acid, DNA, RNA, or cell-free nucleic acid (cfDNA or cfRNA).

[0012] In some examples, the miRNA comprises mature miRNAs and miRNA hairpins.

[0013] In some examples, the signature panel comprises differential expression in 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, or 12 or more miRNAs selected from the group listed in **Tables 1-11**.

[0014] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0015] In some examples, the colon cell proliferative disorder is selected from the group consisting of stage 1 colorectal cancer, stage 2 colorectal cancer, stage 3 colorectal cancer, and stage 4 colorectal cancer.

[0016] In another aspect, the present disclosure provides a classifier capable of distinguishing a population of healthy subjects (e.g., subjects without colon cell proliferative disorder) from subjects with colon cell proliferative disorder, comprising: a) sets of measured values representative of differential miRNA expression in 6 or more, or 12 or more pre-selected miRNAs selected from the group listed in **Tables 1-11**, wherein the measured values are obtained from miRNA expression data from healthy subjects and subjects having a colon cell proliferative disorder, b) wherein the measured values are used to generate a set of features corresponding to properties of the differential miRNA expression, and wherein the set of features is computer processed using machine learning model (e.g., a statistical model), c) wherein the machine model provides a feature vector useful as a classifier capable of distinguishing a population of healthy subjects from subjects having a colon cell proliferative disorder.

[0017] In some examples, the pre-selected miRNAs comprise at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, or at least 250 miRNAs selected from the group listed in **Tables 1-11**.

[0018] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0019] In some examples, the sets of measured values describe characteristics of the differential miRNA expression selected from the group consisting of: count or rate of observing fragments with different counts, raw miRNA abundance, miRNA abundance normalized to

housekeeping genes, miRNA abundance normalized to synthetic sequences, log normalized miRNA abundance, fragment length, fragment midpoint, read mapping position and read pile up along mature miRNAs or miRNA hairpins, and abundances of clusters of miRNAs.

[0020] In some examples, the machine learning model is trained using training data obtained from training biological samples, a first subset of the training biological samples identified as corresponding to a subject having a colon cell proliferative disorder and a second subset of the training biological samples identified corresponding to a subject as not having a colon cell proliferative disorder.

[0021] In some examples, the classifier is provided in a system for detecting a colon cell proliferative disorder comprising: a) a computer-readable medium comprising a classifier operable to classify the subjects based on a miRNA signature panel; and b) one or more processors for executing instructions stored on the computer-readable medium.

[0022] In some examples, the system comprises a classification circuit that is configured as a machine learning classifier selected from the group consisting of a deep learning classifier, a neural network classifier, a linear discriminant analysis (LDA) classifier, a quadratic discriminant analysis (QDA) classifier, a support vector machine (SVM) classifier, a random forest (RF) classifier, a linear kernel support vector machine classifier, a first or second order polynomial kernel support vector machine classifier, a ridge regression classifier, an elastic net algorithm classifier, a sequential minimal optimization algorithm classifier, a naive Bayes algorithm classifier, and principal component analysis classifier.

[0023] In another aspect, the present disclosure provides a method for determining a micro ribonucleic acid (miRNA) profile of a biological sample from a subject, comprising: a) isolating RNA molecules from the biological sample; b) ligating RNA adapters to the RNA molecules, before or after reverse transcribing the RNA molecules to complementary deoxyribonucleic acid (cDNA) molecules; c) amplifying the cDNA molecules; d) determining nucleic acid sequences of the cDNA molecules; e) aligning the nucleic acid sequences to a reference nucleic acid sequence for a panel of miRNAs selected from the group listed in **Tables 1-11**; and f) determining the miRNA profile of the subject based at least in part on the aligned nucleic acid sequences.

[0024] In some examples, the pre-selected miRNAs comprise at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least

110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, or at least 250 miRNAs selected from the group listed in **Tables 1-11**.

[0025] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0026] In some examples, the method further comprises enriching or depleting the RNA molecules or the cDNA molecules.

[0027] In some examples, the reference nucleic acid sequence comprises genome, transcriptome, or custom transcriptome reference nucleic acid sequences.

[0028] In some examples, the method further comprises preparing a miRNA library before the amplifying.

[0029] In some examples, the adapter ligation comprises RNA adapter ligation, adapter blocking, adapter circularization and dimer removal before c).

[0030] In some examples, ligating the RNA adapters comprises performing adapter blocking, adapter circularization, and/or dimer removal.

[0031] In some examples, ligating the RNA adapters comprises performing 3′ RNA adapter ligation, 5′ RNA adapter ligation, reverse transcription with unique molecular identifier (UMI) assignment, and/or cDNA cleanup.

[0032] In another aspect, the present disclosure provides a method for determining a micro ribonucleic acid (miRNA) profile of a biological sample from a subject, comprising performing one or more of: 1) Extraction of RNA molecules from the biological sample followed by direct RNA counting, 2) Extraction of RNA molecules from the biological sample followed by A tailing, then reverse transcribing (RT) to cDNA with template switching, 3) Extraction of RNA molecules from the biological sample followed by A tailing, then reverse transcription polymerase chain reaction (RT-PCR) and quantitative PCR (qPCR) or digital droplet PCR (ddPCR), 4) Extraction of RNA molecules from the biological sample followed by sequence-specific ligation, and then RT-PCR and qPCR or ddPCR, and 5) Extraction-free miRNA profiling of RNA molecules from the biological sample in absence of performing RNA isolation; and determining the miRNA profile of the biological sample from the subject.

[0033] In some examples, determining the miRNA profile comprises use of a reference nucleic acid sequence that is part of a human genome or human transcriptome database.

[0034] In some examples, determining the miRNA profile comprises generating a counts table of expressed miRNA.

[0035] In some examples, determining the miRNA profile comprises generating a counts table normalized based on expressed miRNA to identify differentially-abundant miRNA.

[0036] In some examples, the miRNA profile is associated with a colon cell proliferative disorder and provides classification of a subject as having a colon cell proliferative disorder or not having a colon cell proliferative disorder.

[0037] In some examples, the biological sample from the subject is selected from the group consisting of bodily fluid, stool, colonic effluent, urine, blood plasma, blood serum, whole blood, isolated blood cells, cells isolated from the blood, and combinations thereof.

[0038] In some examples, the method further comprises comparing the miRNA profile against a database of reference miRNA profiles from healthy subjects; and determining that the subject has an increased risk of having a colon cell proliferative disorder based at least in part on measuring a change of at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% in miRNA expression of the miRNA profile relative to the reference miRNA profiles.

[0039] In some examples, the method further comprises comparing the miRNA profile against a database of reference miRNA profiles from healthy subjects; and determining that the subject has an increased risk of having a colon cell proliferative disorder based at least in part on measuring a change of at least 15% in miRNA expression of the miRNA profile relative to the reference miRNA profiles.

[0040] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0041] In some examples, the colon cell proliferative disorder is selected from the group consisting of stage 1 colorectal cancer, stage 2 colorectal cancer, stage 3 colorectal cancer, and stage 4 colorectal cancer.

[0042] In some examples, the advanced adenoma comprises a tubular adenoma, a tubulovillous adenoma, a villous adenoma, an adenocarcinoma, or a hyperplastic polyp.

[0043] In another aspect, the present disclosure provides a method for detecting a presence or an absence of a colon cell proliferative disorder in a subject, comprising: a) isolating ribonucleic acid (RNA) molecules from the biological sample; b) ligating RNA adapters to the RNA molecules, before or after reverse transcribing the RNA molecules to complementary deoxyribonucleic acid (cDNA) molecules; c) amplifying the cDNA molecules; d) determining nucleic acid sequences of the cDNA molecules; e) aligning the nucleic acid sequences to a

reference nucleic acid sequence for a pre-identified panel of miRNAs selected from the group listed in **Tables 1-11**; f) determining an miRNA profile based at least in part on the aligned nucleic acid sequences; and g) computer processing the miRNA profile using a machine learning model trained to be capable of distinguishing between healthy subjects and subjects with a colon cell proliferative disorder to provide an output value associated with presence or absence of a colon cell proliferative disorder, thereby indicating the presence or the absence of the colon cell proliferative disorder in the subject.

[0044] In some examples, b) comprises incorporating sample-specific barcodes and/or molecule-specific unique molecular identifiers (UMIs).

[0045] In some examples, the pre-selected miRNAs comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, or at least 250 miRNAs selected from the group listed in **Tables 1-11**.

[0046] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0047] In some examples, the reference nucleic acid sequence is part of a human genome or human transcriptome database.

[0048] In some examples, determining the miRNA profile of the subject comprises generating a counts table of expressed miRNA.

[0049] In some examples, determining the miRNA profile of the subject comprises generating a counts table of expressed miRNA to identify differentially-abundant miRNA.

[0050] In some examples, the biological sample from the subject is selected from the group consisting of bodily fluid, stool, colonic effluent, urine, blood plasma, blood serum, whole blood, isolated blood cells, cells isolated from the blood, and combinations thereof.

[0051] In some examples, the method further comprises comparing the miRNA profile against a database of reference miRNA profiles from healthy subjects; and determining that the subject has an increased risk of having a colon cell proliferative disorder based at least in part on measuring a change of at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least

30%, at least 35%, at least 40%, at least 45%, or at least 50% in the miRNA expression of the miRNA profile relative to the reference miRNA profiles.

[0052] In some examples, the method further comprises comparing the miRNA profile against a database of reference miRNA profiles from healthy subjects; and determining that the subject has an increased risk of having a colon cell proliferative disorder based at least in part on measuring a change of at least 15% in the miRNA expression of the miRNA profile relative to the reference miRNA profiles.

[0053] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0054] In some examples, the colon cell proliferative disorder is selected from the group consisting of stage 1 colorectal cancer, stage 2 colorectal cancer, stage 3 colorectal cancer, and stage 4 colorectal cancer.

[0055] In another aspect, the present disclosure provides a method for determining a miRNA profile of a biological sample from a subject comprising: a) isolating ribonucleic acid (RNA) molecules from the biological sample; b) reverse transcribing the RNA molecules to complementary deoxyribonucleic acid (cDNA) molecules; c) ligating RNA adapters to the RNA molecules or the cDNA molecules; d) amplifying the cDNA molecules; e) determining nucleic acid sequences of the cDNA molecules; f) aligning the nucleic acid sequences to a reference nucleic acid sequence for a panel of miRNAs selected from the group listed in **Tables 1-11**; and g) determining the miRNA profile based at least in part on the aligned nucleic acid sequences.

[0056] In some examples, the pre-selected miRNAs comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, or at least 250 miRNAs selected from the group listed in **Tables 1-11**.

[0057] In some examples, the method further comprises administering a treatment to the subject based on the detected colon cell proliferative disorder. In some examples, the treatment comprises chemotherapy, radiotherapy, immunotherapy, or surgery.

[0058] In another aspect, the present disclosure provides a method for detecting a presence or an absence of a colon cell proliferative disorder in a subject, comprising: a) isolating ribonucleic acid (RNA) molecules from the biological sample; b) reverse transcribing the RNA molecules to complementary deoxyribonucleic acid (cDNA) molecules; c) ligating RNA adapters to the RNA molecules or the cDNA molecules; d) amplifying the cDNA molecules; e) determining nucleic acid sequences of the cDNA molecules; f) aligning the nucleic acid sequences to a reference nucleic acid sequence for a panel of miRNAs selected from the group listed in **Tables 1-11**; g) determining an miRNA profile based at least in part on the aligned nucleic acid sequences; h) computer processing the miRNA profile using a machine learning model trained to distinguish between subjects not having the colon cell proliferative disorder and subjects having the colon cell proliferative disorder; and i) outputting by the machine learning model a value associated with subjects having the colon cell proliferative disorder or with subjects not having the colon cell proliferative disorder, thereby detecting the presence or the absence of the colon cell proliferative disorder in the subject.

[0059] In some examples, the pre-selected miRNAs comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, or at least 250 miRNAs selected from the group listed in **Tables 1-11**.

[0060] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0061] In some examples, the method further comprises administering a treatment to the subject based on the detected colon cell proliferative disorder. In some examples, the treatment comprises chemotherapy, radiotherapy, immunotherapy, or surgery.

[0062] In another aspect, the present disclosure provides a method for monitoring minimal residual disease in a subject previously treated for a disease, comprising: determining a micro ribonucleic acid (miRNA) profile of a biological sample from the subject using a panel of miRNAs selected from the group listed in **Tables 1-11**, thereby generating a baseline miRNA state; determining a miRNA profile of a biological sample obtained from the subject at one or

more time points after the generating of the baseline miRNA state, thereby generating a current miRNA state; and determining a difference between the baseline miRNA state and the current miRNA state, thereby detecting a change in the minimal residual disease in the subject.

[0063] In some examples, the minimal residual disease is selected from the group consisting of response to treatment, tumor load, residual tumor post-surgery, relapse, secondary screen, primary screen, and cancer progression. In some examples, the method further comprises administering a treatment to the subject based on the detected change in the minimal residual disease in the subject. In some examples, the treatment comprises chemotherapy, radiotherapy, immunotherapy, or surgery.

[0064] In another example, a method is provided for determining response of a subject to treatment, comprising: determining a micro ribonucleic acid (miRNA) profile of a biological sample from the subject using a panel of miRNAs selected from the group listed in **Tables 1-11**, thereby generating a baseline miRNA state; determining a miRNA profile of a biological sample obtained from the subject at one or more time points after the generating of the baseline miRNA state, thereby generating a current miRNA state; and determining a difference between the baseline miRNA state and the current miRNA state, thereby determining the response of the subject to the treatment. In some examples, the method further comprises administering a treatment to the subject based on the determined response of the subject to the treatment. In some examples, the treatment comprises chemotherapy, radiotherapy, immunotherapy, or surgery.

[0065] In another example, a method is provided for monitoring tumor load of a subject, comprising: determining a micro ribonucleic acid (miRNA) profile of a biological sample from the subject using a panel of miRNAs selected from the group listed in **Tables 1-11**, thereby generating a baseline miRNA state; determining a miRNA profile of a biological sample obtained from the subject at one or more time points after the generating of the baseline miRNA state, thereby generating a current miRNA state; and determining a difference between the baseline miRNA state and the current miRNA state, thereby monitoring the tumor load of the subject. In some examples, the method further comprises administering a treatment to the subject based on the tumor load of the subject. In some examples, the treatment comprises chemotherapy, radiotherapy, immunotherapy, or surgery.

[0066] In another example, a method is provided for detecting residual tumor post-surgery of a subject, comprising: determining a micro ribonucleic acid (miRNA) profile of a biological sample from the subject using a panel of miRNAs selected from the group listed in **Tables 1-11**, thereby generating a baseline miRNA state; determining a miRNA profile of a biological sample obtained from the subject at one or more time points after the generating of the baseline miRNA

state, thereby generating a current miRNA state; and determining a difference between the baseline miRNA state and the current miRNA state, thereby detecting the residual tumor post-surgery of the subject. In some examples, the method further comprises administering a treatment to the subject based on the residual tumor post-surgery of the subject. In some examples, the treatment comprises chemotherapy, radiotherapy, immunotherapy, or surgery.

[0067] In another example, a method is provided for detecting relapse of a subject, comprising: determining a micro ribonucleic acid (miRNA) profile of a biological sample from the subject using a panel of miRNAs selected from the group listed in **Tables 1-11**, thereby generating a baseline miRNA state; determining a miRNA profile of a biological sample obtained from the subject at one or more time points after the generating of the baseline miRNA state, thereby generating a current miRNA state; and determining a difference between the baseline miRNA state and the current miRNA state, thereby detecting the relapse of the subject. In some examples, the method further comprises administering a treatment to the subject based on the detected relapse of the subject. In some examples, the treatment comprises chemotherapy, radiotherapy, immunotherapy, or surgery.

[0068] In another example, a method is provided for performing a secondary screen, based at least in part on a miRNA profile of a subject.

[0069] In another example, a method is provided for performing a primary screen, based at least in part on a miRNA profile of a subject.

[0070] In another example, a method is provided for monitoring cancer progression of a subject, comprising: determining a micro ribonucleic acid (miRNA) profile of a biological sample from the subject using a panel of miRNAs selected from the group listed in **Tables 1-11**, thereby generating a baseline miRNA state; determining a miRNA profile of a biological sample obtained from the subject at one or more time points after the generating of the baseline miRNA state, thereby generating a current miRNA state; and determining a difference between the baseline miRNA state and the current miRNA state, thereby monitoring the cancer progression of the subject. In some examples, the method further comprises administering a treatment to the subject based on the cancer progression of the subject. In some examples, the treatment comprises chemotherapy, radiotherapy, immunotherapy, or surgery.

[0071] In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer in the subject at a sensitivity of at least about 40%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer in the subject at a sensitivity of at least about 50%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer in the subject at a sensitivity of at least about 60%. In some examples, the miRNA profile is indicative of a presence or susceptibility of

colorectal cancer in the subject at a sensitivity of at least about 70%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer in the subject at a sensitivity of at least about 80%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer in the subject at a sensitivity of at least about 90%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer in the subject at a sensitivity of at least about 95%.

[0072] In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer in the subject at a positive predictive value (PPV) of at least about 30%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a positive predictive value (PPV) of at least about 40%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a positive predictive value (PPV) of at least about 50%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a positive predictive value (PPV) of at least about 60%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a positive predictive value (PPV) of at least about 70%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a positive predictive value (PPV) of at least about 80%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a positive predictive value (PPV) of at least about 90%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a positive predictive value (PPV) of at least about 95%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a positive predictive value (PPV) of at least about 99%.

[0073] In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a negative predictive value (NPV) of at least about 40%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a negative predictive value (NPV) of at least about 50%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a negative predictive value (NPV) of at least about 60%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a negative predictive value (NPV) of at least about 70%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a negative predictive value (NPV) of at least about 80%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a negative predictive value (NPV) of at least about 90%. In some examples, the miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a negative predictive value (NPV) of at least about 95%. In some examples, the

miRNA profile is indicative of a presence or susceptibility of colorectal cancer at a negative predictive value (NPV) of at least about 99%.

[0074] In some examples, the trained algorithm determines a presence or susceptibility of colorectal cancer of the subject with an Area Under Curve (AUC) of at least about 0.50. In some examples, the trained algorithm determines a presence or susceptibility of colorectal cancer of the subject with an Area Under Curve (AUC) of at least about 0.60. In some examples, the trained algorithm determines a presence or susceptibility of colorectal cancer of the subject with an Area Under Curve (AUC) of at least about 0.70. In some examples, the trained algorithm determines a presence or susceptibility of colorectal cancer of the subject with an Area Under Curve (AUC) of at least about 0.80. In some examples, the trained algorithm determines a presence or susceptibility of colorectal cancer of the subject with an Area Under Curve (AUC) of at least about 0.90. In some examples, the trained algorithm determines a presence or susceptibility of colorectal cancer of the subject with an Area Under Curve (AUC) of at least about 0.95. In some examples, the trained algorithm determines a presence or susceptibility of colorectal cancer of the subject with an Area Under Curve (AUC) of at least about 0.99.

[0075] In some examples, the method further comprises presenting a report or a graphical user interface of an electronic device of a user. In some examples, the user is the subject, individual, or patient.

[0076] In some examples, the method further comprises determining a likelihood of the determination of a presence or susceptibility of colorectal cancer in the subject, individual, or patient.

[0077] In some examples, the trained algorithm (e.g., machine learning model or classifier) comprises a supervised machine learning algorithm. In some examples, the supervised machine learning algorithm comprises a deep learning algorithm, a support vector machine (SVM), a neural network, or a Random Forest.

[0078] In some examples, the method further comprises providing the subject with a therapeutic intervention based at least in part on the miRNA profile or analysis, such as a therapeutic intervention to treat a patient with colorectal cancer (e.g., chemotherapy, radiotherapy, immunotherapy, or surgery).

[0079] In some examples, the method further comprises monitoring the presence or susceptibility of the colorectal cancer, wherein the monitoring comprises assessing the presence or susceptibility of the colorectal cancer of said subject at a plurality of time points, wherein the assessing is based at least on the presence or susceptibility of the colorectal cancer determined at each of the plurality of time points.

[0080] In some examples, a difference in the assessment of the presence or susceptibility of the colorectal cancer of the subject among the plurality of time points is indicative of one or more clinical indications selected from the group consisting of: (i) a diagnosis of the presence or susceptibility of the colorectal cancer of the subject, (ii) a prognosis of the presence or susceptibility of the colorectal cancer of the subject, and (iii) an efficacy or non-efficacy of a course of treatment for treating the presence or susceptibility of the colorectal cancer of the subject.

[0081] In some examples, the method further comprises stratifying the colorectal cancer of the subject by using the trained algorithm to determine a sub-type of the colorectal cancer of the subject from among a plurality of distinct subtypes or stages of colorectal cancer.

[0082] Another example of the present disclosure provides a classifier for distinguishing a population of subjects having a colon cell proliferative disorder from subjects not having the colon cell proliferative disorder comprising: sets of measured values representative of differential miRNA abundance in 6 or more miRNAs selected from the group listed in **Tables 1-11**, wherein the measured values are obtained from miRNA expression data from subjects not having the colon cell proliferative disorder and subjects having the colon cell proliferative disorder, wherein the measured values are used to generate a set of features corresponding to properties of the differential miRNA abundance and wherein the features are incorporated into a machine learning or statistical model, wherein the machine learning or statistical model provides a feature vector useful as a classifier capable of distinguishing a population of subjects not having the colon cell proliferative disorder from subjects having a colon cell proliferative disorder.

[0083] Another example of the present disclosure provides a non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0084] Another example of the present disclosure provides a system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0085] Another example of the present disclosure provides a system comprising: a) a computer-readable medium comprising a classifier for distinguishing a population of subjects having a colon cell proliferative disorder from subjects not having the colon cell proliferative disorder based on a miRNA signature panel using a machine learning model; and b) one or more processors for executing instructions stored on the computer-readable medium.