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(54) PROBE FOR LEUKEMIA DETECTION AND DESIGN METHOD THEREOF.

(57) The present invention belongs to the technical field of leukemia detection, discloses a probe for leukemia detection and a design method thereof. The present invention has high sensitivity, high specificity: the use of multiple aptamer design can effectively improve the sensitivity, specificity of leukemia cell detection, especially showing superiority in early detection. Rapid detection and real-time monitoring: The new probe has the ability of rapid detection, is suitable for instant detection, clinical real-time monitoring. Multiple marker analysis: Through multiple detections, a comprehensive analysis of leukemia cells can be achieved, providing more pathological information, helping to optimize treatment plans. Biocompatibility and stability: The use of nanocarriers improves the stability, service life of the probe in the biological environment, ensuring safety and reliability in clinical applications. The present invention provides a new leukemia detection probe with significant technical advantages, which provides a powerful tool for the early diagnosis and treatment of leukemia.

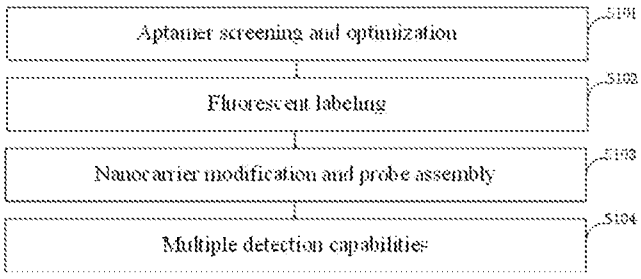

FIG. 1

DESCRIPTION

LU508286

PROBE FOR LEUKEMIA DETECTION AND DESIGN METHOD THEREOF

FIELD OF TECHNOLOGY

[0001] The invention belongs to the technical field of leukemia detection, and in particular relates to a probe for leukemia detection and a design method thereof.

BACKGROUND

[0002] In the field of leukemia detection, traditional methods mainly rely on morphological cell analysis, immunofluorescence staining, flow cytometry and other technologies. Although these methods can identify and classify leukemia cells, they are complex to operate, time-consuming, and have limited sensitivity and specificity. In addition, nucleic acid amplification-based technologies (such as PCR) are also widely used in leukemia detection, which can diagnose the disease by detecting specific gene mutations or fusion genes. However, these methods still have problems such as insufficient sensitivity, high false positive rate, and difficulty in detecting leukemia cells in the early stages.

[0003] Through the above analysis, the problems and defects of the prior art are as follows:

[0004] (1) Insufficient sensitivity and specificity: The sensitivity and specificity of existing detection methods may be insufficient, making it difficult to detect leukemia cells at an early stage or identify low-frequency mutations.

[0005] (2) Complex and time-consuming operations: Existing methods usually require complex operations and long detection times, which is not conducive to rapid diagnosis.

[0006] (3) High false positive rate: Existing methods may lead to false positive results due to nonspecific binding or contamination problems, thus affecting diagnostic accuracy.

[0007] (4) Difficulty in real-time monitoring: Most existing detection methods are endpoint detection, which makes it difficult to monitor leukemia cell changes in real time.

SUMMARY

[0008] In view of the problems existing in the prior art, the present invention provides a probe for leukemia detection and a design method.

[0009] The present invention is implemented in such a way that a probe for leukemia detection and a design method thereof include:

[0010] Step 1, aptamer screening and optimization:

[0011] The nucleic acid aptamers that specifically bind to leukemia cell surface markers were screened out through SELEX technology; the aptamers have high affinity and specificity, and recognize and bind to leukemia cells without non-specific binding to normal cells;

[0012] Step 2, fluorescent labeling:

[0013] The selected aptamers are labeled with fluorescent molecules to form fluorescent probes; the fluorescent markers emit fluorescent signals after the aptamers bind to the surface markers of leukemia cells;

[0014] Step 3, Nanocarrier modification and probe assembly:

[0015] The fluorescently labeled aptamers were modified on the nanocarriers to enhance their stability and biocompatibility;

[0016] Step 4, multiple detection capabilities:

[0017] By adjusting the sequence and fluorescent markers of the aptamer, multiplex detection can be performed, that is, multiple leukemia cell markers can be identified simultaneously.

[0018] In combination with the above technical solutions and the technical problems solved, the advantages and positive effects of the technical solutions to be protected by the present invention are as follows:

[0019] The present invention has high sensitivity and high specificity: the probe specifically recognizes leukemia cell surface markers through aptamers, avoids nonspecific binding, improves the sensitivity and specificity of detection, and can detect leukemia at an early stage.

[0020] Easy and fast operation: The probe can be used on simple operating platforms, such as fluorescence microscopes or flow cytometers, and the detection results can be quickly obtained through fluorescence signals, shortening the detection time.

[0021] Reduce false positive rate: Due to the high specific recognition ability of the aptamer, nonspecific binding is reduced, thereby significantly reducing the false positive rate and improving the accuracy of diagnosis.

[0022] Real-time monitoring capability: The fluorescent signal of the probe can be used to monitor leukemia cells in real time, making it easier for clinicians to dynamically observe changes in the patient's condition and adjust treatment plans in a timely manner.

[0023] The application of this probe provides an efficient, accurate and convenient new tool for the early diagnosis and real-time monitoring of leukemia, significantly improving the shortcomings of existing technologies.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] FIG. 1 is a flow chart of a probe and design method for leukemia detection provided in an embodiment of the present invention.

[0025] FIG. 2 is a flow chart of a method for screening and optimizing nucleic acid aptamers provided in an embodiment of the present invention.

[0026] FIG. 3 is a structural block diagram of a probe design system for leukemia detection provided by an embodiment of the present invention.

DESCRIPTION OF THE EMBODIMENTS

[0027] As shown in FIG. 1, a probe and design method for leukemia detection provided by an embodiment of the present invention includes the following steps:

[0028] S101, nucleic acid aptamer screening and optimization:

[0029] The nucleic acid aptamers that specifically bind to leukemia cell surface markers were screened out through SELEX technology; the aptamers have high affinity and specificity, and recognize and bind to leukemia cells without non-specific binding to normal cells;

[0030] S102, fluorescent labeling:

[0031] The selected aptamers are labeled with fluorescent molecules to form fluorescent probes; the fluorescent markers emit fluorescent signals after the aptamers bind to the surface markers of leukemia cells;

[0032] S103, Nanocarrier modification and probe assembly:

[0033] The fluorescently labeled aptamers were modified on the nanocarriers to enhance their stability and biocompatibility;

[0034] S104, multiple detection capabilities:

[0035] By adjusting the sequence and fluorescent markers of the aptamer, multiplex detection can be performed, that is, multiple leukemia cell markers can be identified simultaneously.

[0036] The probe for leukemia detection and the design method thereof provided in the embodiment of the present invention realize efficient and specific leukemia cell detection through a series of precise steps. The design and working principle of the probe can be described in detail in the following four parts:

[0037] 1. Screening and optimization of nucleic acid aptamers:

[0038] First, nucleic acid aptamers that can specifically bind to leukemia cell surface markers are screened from a large number of random nucleic acid sequence libraries through the systematic evolution ligand exponential enrichment technology (SELEX). These aptamers have high affinity and specificity, and can accurately identify and bind to specific markers on the surface of leukemia cells without non-specific binding to normal cells.

[0039] The key to this step is to ensure that the aptamers have sufficient sensitivity and specificity in actual detection through repeated screening and optimization.

[0040] 2. Fluorescent labeling:

[0041] Next, the selected nucleic acid aptamers are chemically labeled with fluorescent molecules to form fluorescent probes. When the aptamers bind to the surface markers of leukemia cells, the fluorescent markers will emit specific fluorescent signals, thereby realizing the detection of leukemia cells. The emission of this fluorescent signal is a key indicator in the detection process. By detecting the intensity and position of the fluorescent signal, the presence and distribution of leukemia cells can be directly determined.

[0042] 3. Nanocarrier modification and probe assembly:

[0043] In order to enhance the stability and biocompatibility of the probe, the fluorescently labeled aptamer is modified on the nanocarrier. The nanocarrier not only provides the fixation and protection of the aptamer, but also improves the stability of the probe in the biological system and prevents the degradation of the aptamer in the in vivo or in vitro detection environment. In addition, the presence of the nanocarrier can also increase the surface area of the probe, thereby increasing the contact probability between the probe and the target cell, further enhancing the sensitivity of the detection.

[0044] 4. Multiple detection capabilities:

[0045] Finally, by adjusting the sequence of the aptamer and the type of fluorescent marker, a multifunctional probe that can simultaneously identify multiple leukemia cell markers can be designed. This multiplex detection capability enables the probe to not only detect a single marker, but also to simultaneously identify and quantitatively analyze multiple leukemia-related markers, thereby providing more comprehensive and accurate leukemia cell detection results. This multiplex detection greatly improves detection efficiency and accuracy, and is particularly suitable for clinical diagnosis and research of complex conditions.

[0046] As shown in FIG. 2, the nucleic acid aptamer screening and optimization method provided by the embodiment of the present invention is as follows:

[0047] S201, initial screening

[0048] Using SELEX technology, nucleic acid aptamers with high affinity for specific leukemia cell surface markers were initially screened; including multiple rounds of binding, separation and enrichment steps to screen the optimal aptamer sequence from a random oligonucleotide library;

[0049] S202, aptamer sequence optimization

[0050] The screened aptamer sequences were further structurally optimized, and chemical modifications were introduced to enhance the aptamer's resistance to enzyme degradation and binding stability.

[0051] The fluorescent label provided by the embodiment of the present invention is:

[0052] (1) Fluorescent molecular labeling

[0053] Select appropriate fluorescent dyes and label the fluorescent molecules on the 5' end or 3' end of the optimized aptamer through covalent bonds to prepare fluorescent labeled probes;

[0054] (2) Probe verification

[0055] The specificity and sensitivity of the fluorescently labeled probes were verified through in vitro experiments to ensure that the probes could specifically recognize and bind to target leukemia cells and emit strong fluorescent signals after binding.

[0056] Nanocarrier modification and probe assembly provided by the embodiments of the present invention:

[0057] 1) Selection and preparation of nanocarriers

[0058] Selecting a suitable nanocarrier material with active groups on its surface to which the fluorescently labeled aptamer can bind;

[0059] 2) Modification and assembly of fluorescent probes

[0060] The fluorescently labeled aptamer is modified on the surface of the nanocarrier by chemical bonding or physical adsorption to form a nano fluorescent probe complex; the surface density of the aptamer should be controlled during the modification process to ensure that each nanocarrier carries a sufficient number of aptamers;

[0061] 3) Functional verification of the probe

[0062] The functions of the nanofluorescent probe, including its stability in complex biological environments and its ability to specifically bind to target leukemia cells, were verified through biocompatibility testing and in vitro and in vivo specific recognition experiments.

[0063] The multiple detection capabilities provided by the embodiments of the present invention are:

[0064] 1) Multiple aptamer design

[0065] Different nucleic acid aptamers are designed for various markers on the surface of leukemia cells, and different fluorescent molecules are labeled respectively; each fluorescent molecule should have different excitation and emission spectra;

[0066] 2) Multiple probe combinations

[0067] A multifunctional nano fluorescent probe is prepared by combining a variety of fluorescently labeled aptamers with nanocarriers. The probe can simultaneously identify multiple leukemia cell markers and perform multicolor fluorescence analysis through fluorescence microscopy or flow cytometry to achieve simultaneous detection of different cell subtypes or multiple markers.

[0068] 3) Data analysis and identification.

[0069] Data analysis and identification provided by the embodiments of the present invention:

[0070] Dedicated software or algorithms are used to analyze and interpret the fluorescent signals obtained from multiple tests to distinguish different types of leukemia cells or identify cell subpopulations with different marker expression patterns.

[0071] As shown in FIG. 3, a probe design system for leukemia detection provided by an embodiment of the present invention includes:

[0072] A screening module is used to screen nucleic acid aptamers that specifically bind to leukemia cell surface markers through SELEX technology; aptamers have high affinity and specificity, recognize and bind to leukemia cells without non-specific binding to normal cells;

[0073] The fluorescent labeling module is used to label the selected aptamers with fluorescent molecules to form fluorescent probes; the fluorescent labeling emits a fluorescent signal after the aptamer binds to the leukemia cell surface marker;

[0074] A modification module, used to modify the fluorescently labeled aptamer on the nanocarrier to enhance its stability and biocompatibility;

[0075] The multiplex detection module is used to perform multiplex detection by adjusting the sequence of the aptamer and the fluorescent marker, that is, to simultaneously identify multiple leukemia cell markers.

[0076] This protocol proposes a new probe for leukemia detection. The probe is based on aptamer technology and can identify specific markers on the surface of leukemia cells with high specificity. The design of the probe includes the following steps:

[0077] 1. Screening of nucleic acid aptamers: nucleic acid aptamers that specifically bind to leukemia cell surface markers are screened through SELEX technology. Aptamers have high affinity and specificity, and can recognize and bind to leukemia cells without non-specific binding to normal cells.

[0078] 2. Fluorescent labeling: The selected aptamers are labeled with fluorescent molecules to form fluorescent probes. Fluorescent labeling can emit fluorescent signals after the aptamers bind to leukemia cell surface markers, making it easier to detect.

[0079] 3. Nanocarrier modification: The fluorescently labeled aptamer is modified on the nanocarrier to enhance its stability and biocompatibility. The use of nanocarriers can improve the stability of the probe in the biological system and reduce nonspecific adsorption.

[0080] 4. Multiplex detection capability: The probe can perform multiplex detection by adjusting the sequence of the aptamer and the fluorescent marker, that is, simultaneously identifying multiple leukemia cell markers to improve the comprehensiveness and accuracy of the detection.

[0081] In order to realize the above technical solution, the present invention provides the following specific implementation scheme to ensure the efficiency and practicality of the new probe:

[0082] 1. Screening and optimization of nucleic acid aptamers

[0083] Step 1: Initial Screening

[0084] Using the SELEX (SystematicEvolutionofLigandsbExponentialEnrichment) technique, nucleic acid aptamers with high affinity for specific leukemia cell surface markers (such as CD33, CD123, etc.) were initially screened. The process includes multiple rounds of binding, separation, and enrichment steps to screen out the optimal aptamer sequence from a random oligonucleotide library.

[0085] Step 2: Aptamer sequence optimization

[0086] In order to improve the specificity and stability of the aptamer, the screened aptamer sequence can be further structurally optimized, such as by introducing chemical modifications (such as 2'-fluorine modification, phosphorothioate bond, etc.) to enhance the aptamer's resistance to enzyme degradation and binding stability.

[0087] 2. Preparation of Fluorescently Labeled Probes

[0088] Step 1: Fluorescent Molecular Labeling

[0089] Select appropriate fluorescent dyes (such as FITC, Cy3, Cy5, etc.), label the fluorescent molecules on the 5' or 3' end of the optimized aptamer through covalent bonds to prepare fluorescent labeled probes. The selection of labeling position and fluorescent dye should take into account fluorescence intensity, photostability and biocompatibility.

[0090] Step 2: Probe Verification

[0091] The specificity and sensitivity of the fluorescently labeled probes are verified through in vitro experiments (such as fluorescence microscopy observation or flow cytometry detection) to ensure that the probes can specifically recognize and bind to target leukemia cells and emit strong fluorescent signals after binding.

[0092] 3. Nanocarrier modification and probe assembly

[0093] Step 1: Selection and preparation of nanocarriers

[0094] Select appropriate nanocarrier materials (such as gold nanoparticles, silica nanoparticles or polymer nanoparticles) with active groups (such as amino groups, thiol groups, etc.) on their surfaces for the fluorescently labeled aptamers to bind to. These nanocarriers need to have good biocompatibility and stability in vivo.

[0095] Step 2: Modification and assembly of fluorescent probes

[0096] The fluorescently labeled aptamer is modified on the surface of the nanocarrier by chemical bonding or physical adsorption to form a nano fluorescent probe complex. The surface density of the aptamer should be controlled during the modification process to ensure that each nanocarrier carries a sufficient number of aptamers to improve the detection sensitivity of the probe.

[0097] Step 3: Functional verification of the probe

[0098] The functions of the nanofluorescent probe were verified through biocompatibility testing, in vivo and in vitro specific recognition experiments, etc., including its stability in complex biological environments and its ability to specifically bind to target leukemia cells.

[0099] 4. Realization of multiple detection capabilities

[0100] Step 1: Multiplex aptamer design

[0101] Different nucleic acid aptamers are designed for various markers on the surface of leukemia cells (such as CD19, CD20, CD33, etc.), and are labeled with different fluorescent molecules. Each fluorescent molecule should have different excitation and emission spectra to facilitate simultaneous detection.

[0102] Step 2: Multiplex probe panel

[0103] A variety of fluorescently labeled aptamers are combined with nanocarriers to prepare multifunctional nano fluorescent probes. The probes can simultaneously identify multiple leukemia cell markers, and perform multicolor fluorescence analysis through fluorescence microscopy or flow cytometry to achieve simultaneous detection of different cell subtypes or multiple markers.

[0104] Step 3: Data Analysis and Identification

[0105] Using dedicated software or algorithms, the fluorescent signals obtained from multiple tests are analyzed and interpreted to distinguish different types of leukemia cells or identify cell subpopulations with different marker expression patterns. This solution can significantly improve the accuracy and information content of the test.

[0106] The following is a detailed implementation scheme for developing and applying the leukemia detection probe described in the present invention.

[0107] Implementation: Leukemia cell detection probe based on fluorescently labeled nucleic acid aptamers

[0108] 1. Screening and optimization of nucleic acid aptamers

[0109] nucleic acid aptamers that specifically bind to leukemia cell surface markers (such as CD33, CD34, etc.).

[0110] 1) Prepare a random nucleic acid sequence library: Synthesize a nucleic acid library containing approximately 10^15 random sequences with a sequence length of 40-80 bases and known primer sites at both ends of the sequence.

[0111] 2) SELEX screening:

[0112] The nucleic acid sequence library is incubated with leukemia cells (such as K562 cells), and the unbound nucleic acid sequences are removed by elution.

[0113] The bound nucleic acid sequences are amplified by PCR and subjected to a new round of screening. After multiple rounds of screening, nucleic acid sequences with strong binding and high specificity are selected.

[0114] 3) Optimization: Further improve the stability and specificity of the aptamer through chemical modification (such as 5' terminal amino modification) and sequence optimization.

[0115] 2. Fluorescent labeling

[0116] Objective: To label the selected nucleic acid aptamers with fluorescent molecules to form fluorescent probes.

[0117] Step:

[0118] 1) Select fluorescent markers: Select appropriate fluorescent molecules (such as FITC, Cy5, etc.) to label the aptamer.

[0119] 2) Chemical labeling:

[0120] An amino group or a thiol group is introduced at the 5' or 3' end of the aptamer, and these active groups are used to covalently bind to the active groups of the fluorescent molecule (such as NHS ester or maleimide).

[0121] 3) Purification: The labeled aptamers are purified by HPLC or gel electrophoresis to remove unreacted fluorescent molecules and other impurities.

[0122] 3. Nanocarrier modification and probe assembly

[0123] Fluorescently labeled aptamers were immobilized on nanocarriers to improve the stability and detection sensitivity of the probes.

[0124] 1) Select nanocarriers: Choose nanomaterials suitable for biological applications, such as gold nanoparticles, magnetic nanoparticles, or silica nanoparticles.

[0125] 2) Surface modification:

[0126] The surface of the nanocarrier is modified, such as by introducing a carboxyl group, an amino group or a thiol group, so that it can be covalently bound to the fluorescently labeled aptamer.

[0127] 3) Probe assembly:

[0128] The fluorescently labeled aptamer reacts with the modified nanocarrier to form a stable nanoprobe.

[0129] The unbound aptamers were removed by centrifugation and washing, and finally the functionalized probes were obtained.

[0130] 4. Multiple detection capabilities

[0131] Objective: To achieve simultaneous detection of multiple markers on the surface of leukemia cells and improve the accuracy and comprehensiveness of detection.

[0132] Step:

[0133] 1) Design of multiple aptamers: Based on the different surface markers of leukemia cells (such as CD33, CD34, CD45, etc.), multiple nucleic acid aptamers are designed, and each aptamer is combined with a different fluorescent marker (such as FITC, Cy3, Cy5).

[0134] 2) Assembling multifunctional probes: aptamers with different fluorescent labels are modified on different nanocarriers respectively, or multiple modifications are performed on the same nanocarrier to achieve simultaneous detection of multiple markers.

[0135] 3) Detection and analysis:

[0136] In actual detection, the probe is incubated with cells in the sample, and the fluorescent signal can be detected by fluorescence microscopy or flow cytometry.

[0137] By analyzing different fluorescent signals, comprehensive detection of leukemia cells can be achieved.

[0138] The probe can be applied to blood samples of clinical leukemia patients to detect the expression of multiple leukemia markers by flow cytometry, providing rapid and sensitive leukemia diagnosis. In addition, the probe can also be used to study the pathological mechanism of leukemia, screen new drug targets, and provide a basis for personalized treatment.

[0139] Through the above steps, a highly sensitive and specific leukemia detection probe was developed. The probe can realize the simultaneous detection of multiple markers and has good biocompatibility and stability, which is suitable for a variety of application scenarios in clinical diagnosis and research.

CLAIMS

1. A probe for leukemia detection and a design method thereof, characterized in that it comprises the following steps:

step 1, aptamer screening and optimization:

the nucleic acid aptamers that specifically bind to leukemia cell surface markers were screened out through SELEX technology; the aptamers have high affinity and specificity, and recognize and bind to leukemia cells without non-specific binding to normal cells;

step 2, fluorescent labeling:

the selected aptamers are labeled with fluorescent molecules to form fluorescent probes; the fluorescent markers emit fluorescent signals after the aptamers bind to the surface markers of leukemia cells;

step 3, Nanocarrier modification and probe assembly:

the fluorescently labeled aptamers were modified on the nanocarriers to enhance their stability and biocompatibility;

step 4, multiple detection capabilities:

by adjusting the sequence and fluorescent markers of the aptamer, multiplex detection can be performed, that is, multiple leukemia cell markers can be identified simultaneously.

2. The probe for leukemia detection and the design method thereof according to claim 1, characterized in that the nucleic acid aptamer screening and optimization method is as follows:

(1) preliminary screening

using SELEX technology, nucleic acid aptamers with high affinity for specific leukemia cell surface markers were initially screened; including multiple rounds of binding, separation and enrichment steps to screen the optimal aptamer sequence from a random oligonucleotide library;

(2) aptamer sequence optimization

the screened aptamer sequences were further structurally optimized, and chemical modifications were introduced to enhance the aptamer's resistance to enzyme

degradation and binding stability.

3. The probe for leukemia detection and the design method thereof according to claim 1, wherein the fluorescent marker:

(1) fluorescent molecular labeling

select appropriate fluorescent dyes and label the fluorescent molecules on the 5' end or 3' end of the optimized aptamer through covalent bonds to prepare fluorescent labeled probes;

(2) probe verification

the specificity and sensitivity of the fluorescent-labeled probe were verified through in vitro experiments to ensure that the probe could specifically recognize and bind to the target leukemia cells and emit a strong fluorescent signal after binding.

4. The probe for leukemia detection and the design method thereof according to claim 1, characterized in that the nanocarrier modification and probe assembly:

1) selection and preparation of nanocarriers

selecting a suitable nanocarrier material with active groups on its surface to which the fluorescently labeled aptamer can bind;

2) modification and assembly of fluorescent probes

the fluorescently labeled aptamer is modified on the surface of the nanocarrier by chemical bonding or physical adsorption to form a nano fluorescent probe complex; the surface density of the aptamer should be controlled during the modification process to ensure that each nanocarrier carries a sufficient number of aptamers;

3) functional verification of the probe

the functions of the nanofluorescent probe, including its stability in complex biological environments and its ability to specifically bind to target leukemia cells, were verified through biocompatibility testing and in vivo and in vitro specific recognition experiments.

5. The probe for leukemia detection and the design method thereof according to claim 1, wherein the multiple detection capability:

1) multiple aptamer design

different nucleic acid aptamers are designed for various markers on the surface of leukemia cells, and different fluorescent molecules are labeled respectively; each fluorescent molecule should have different excitation and emission spectra;

2) multiple probe combinations

a multifunctional nano fluorescent probe is prepared by combining a variety of fluorescently labeled aptamers with nanocarriers; the probe can simultaneously identify multiple leukemia cell markers and perform multicolor fluorescence analysis through fluorescence microscopy or flow cytometry to achieve simultaneous detection of different cell subtypes or multiple markers;

3) data analysis and identification.

6. The probe for leukemia detection and the design method thereof according to claim 5, characterized in that the data analysis and identification:

dedicated software or algorithms are used to analyze and interpret the fluorescent signals obtained from multiple tests to distinguish different types of leukemia cells or identify cell subpopulations with different marker expression patterns.

7. A probe design system for leukemia detection implementing the probe for leukemia detection and the design method thereof according to any one of claims 1 to 6, characterized in that the probe design system for leukemia detection comprises:

a screening module is used to screen nucleic acid aptamers that specifically bind to leukemia cell surface markers through SELEX technology; aptamers have high affinity and specificity, recognize and bind to leukemia cells without non-specific binding to normal cells;

the fluorescent labeling module is used to label the selected aptamers with fluorescent molecules to form fluorescent probes; the fluorescent labeling emits a fluorescent signal after the aptamer binds to the leukemia cell surface marker;

a modification module, used to modify the fluorescently labeled aptamer on the

nanocarrier to enhance its stability and biocompatibility;

the multiplex detection module is used to perform multiplex detection by adjusting the sequence of the aptamer and the fluorescent marker, that is, to simultaneously identify multiple leukemia cell markers.

REVENDICATIONS

1. Sonde de détection de leucémie et procédé de conception de celle-ci, caractérisée en ce qu'elle comprend les étapes suivantes :

étape 1, criblage et optimisation d'aptamères :

les aptamères d'acide nucléique qui se lient spécifiquement aux marqueurs de surface des cellules leucémiques ont été criblés par la technologie SELEX ; les aptamères ont une affinité et une spécificité élevées, et reconnaissent et se lient aux cellules leucémiques sans liaison non spécifique aux cellules normales ;

étape 2, marquage fluorescent :

les aptamères sélectionnés sont marqués avec des molécules fluorescentes pour former des sondes fluorescentes ; les marqueurs fluorescents émettent des signaux fluorescents après que les aptamères se lient aux marqueurs de surface des cellules leucémiques ;

étape 3, modification du nanotransporteur et assemblage de la sonde :

les aptamères marqués par fluorescence ont été modifiés sur les nanotransporteurs pour améliorer leur stabilité et leur biocompatibilité ;

étape 4, capacités de détection multiples :

en ajustant la séquence et les marqueurs fluorescents de l'aptamère, une détection multiplex peut être effectuée, c'est-à-dire que plusieurs marqueurs de cellules leucémiques peuvent être identifiés simultanément.

2. Sonde de détection de leucémie et son procédé de conception selon la revendication 1, caractérisée en ce que le procédé de criblage et d'optimisation d'aptamères d'acide nucléique est le suivant :

(1) criblage préliminaire

en utilisant la technologie SELEX, des aptamères d'acide nucléique ayant une affinité élevée pour des marqueurs de surface cellulaire leucémique spécifiques ont été initialement criblés ; comprenant plusieurs cycles d'étapes de liaison, de séparation et d'enrichissement pour cribler la séquence d'aptamères optimale à partir d'une

bibliothèque d'oligonucléotides aléatoires ;

(2) optimisation de la séquence d'aptamères

les séquences d'aptamères criblées ont été encore optimisées structurellement et des modifications chimiques ont été introduites pour améliorer la résistance de l'aptamère à la dégradation enzymatique et la stabilité de liaison.

3. Sonde de détection de leucémie et son procédé de conception selon la revendication 1, dans laquelle le marqueur fluorescent :

(1) marquage moléculaire fluorescent

sélectionner des colorants fluorescents appropriés et marquer les molécules fluorescentes sur l'extrémité 5' ou l'extrémité 3' de l'aptamère optimisé par des liaisons covalentes pour préparer des sondes marquées par fluorescence ;

(2) vérification de la sonde

la spécificité et la sensibilité de la sonde marquée par fluorescence ont été vérifiées par des expériences in vitro pour garantir que la sonde pouvait reconnaître et se lier spécifiquement aux cellules leucémiques cibles et émettre un signal fluorescent puissant après la liaison.

4. Sonde de détection de leucémie et procédé de conception de celle-ci selon la revendication 1, caractérisée en ce que la modification du nanotransporteur et l'assemblage de la sonde :

1) sélection et préparation des nanotransporteurs

sélection d'un matériau nanotransporteur approprié avec des groupes actifs sur sa surface auxquels l'aptamère marqué par fluorescence peut se lier ;

2) modification et assemblage de sondes fluorescentes

l'aptamère marqué par fluorescence est modifié sur la surface du nanotransporteur par liaison chimique ou adsorption physique pour former un complexe de sondes nanofluorescentes ; la densité de surface de l'aptamère doit être contrôlée pendant le processus de modification pour garantir que chaque nanotransporteur porte un nombre suffisant d'aptamères ;

3) vérification fonctionnelle de la sonde

les fonctions de la sonde nanofluorescente, y compris sa stabilité dans des environnements biologiques complexes et sa capacité à se lier spécifiquement aux cellules leucémiques cibles, ont été vérifiées par des tests de biocompatibilité et des expériences de reconnaissance spécifique in vivo et in vitro.

5. Sonde de détection de leucémie et procédé de conception de celle-ci selon la revendication 1, dans laquelle la capacité de détection multiple :

1) conception d'aptamères multiples

différents aptamères d'acide nucléique sont conçus pour divers marqueurs à la surface des cellules leucémiques, et différentes molécules fluorescentes sont respectivement marquées ; chaque molécule fluorescente doit avoir des spectres d'excitation et d'émission différents ;

2) combinaisons de sondes multiples

une sonde nanofluorescente multifonctionnelle est préparée en combinant une variété d'aptamères marqués par fluorescence avec des nanotransporteurs ; la sonde peut identifier simultanément plusieurs marqueurs de cellules leucémiques et effectuer une analyse de fluorescence multicolore par microscopie à fluorescence ou cytométrie de flux pour obtenir une détection simultanée de différents sous-types de cellules ou de plusieurs marqueurs ;

3) analyse et identification des données.

6. Sonde de détection de leucémie et son procédé de conception selon la revendication 5, caractérisée en ce que l'analyse et l'identification des données :

un logiciel ou des algorithmes dédiés sont utilisés pour analyser et interpréter les signaux fluorescents obtenus à partir de multiples tests afin de distinguer différents types de cellules leucémiques ou d'identifier des sous-populations cellulaires présentant différents profils d'expression de marqueurs.

7. Système de conception de sonde pour la détection de leucémie mettant en œuvre la sonde de détection de leucémie et son procédé de conception selon l'une quelconque des revendications 1 à 6, caractérisé en ce que le système de conception de sonde pour la détection de leucémie comprend :

un module de criblage est utilisé pour cribler des aptamères d'acide nucléique qui se lient spécifiquement aux marqueurs de surface des cellules leucémiques par la technologie SELEX ; les aptamères ont une affinité et une spécificité élevées, reconnaissent et se lient aux cellules leucémiques sans liaison non spécifique aux cellules normales ;

le module de marquage fluorescent est utilisé pour marquer les aptamères sélectionnés avec des molécules fluorescentes pour former des sondes fluorescentes ; le marquage fluorescent émet un signal fluorescent après que l'aptamère se lie au marqueur de surface des cellules leucémiques ;

un module de modification, utilisé pour modifier l'aptamère marqué par fluorescence sur le nanotransporteur afin d'améliorer sa stabilité et sa biocompatibilité ;

le module de détection multiplex est utilisé pour effectuer une détection multiplex en ajustant la séquence de l'aptamère et du marqueur fluorescent, c'est-à-dire pour identifier simultanément plusieurs marqueurs de cellules leucémiques.

FIGURES

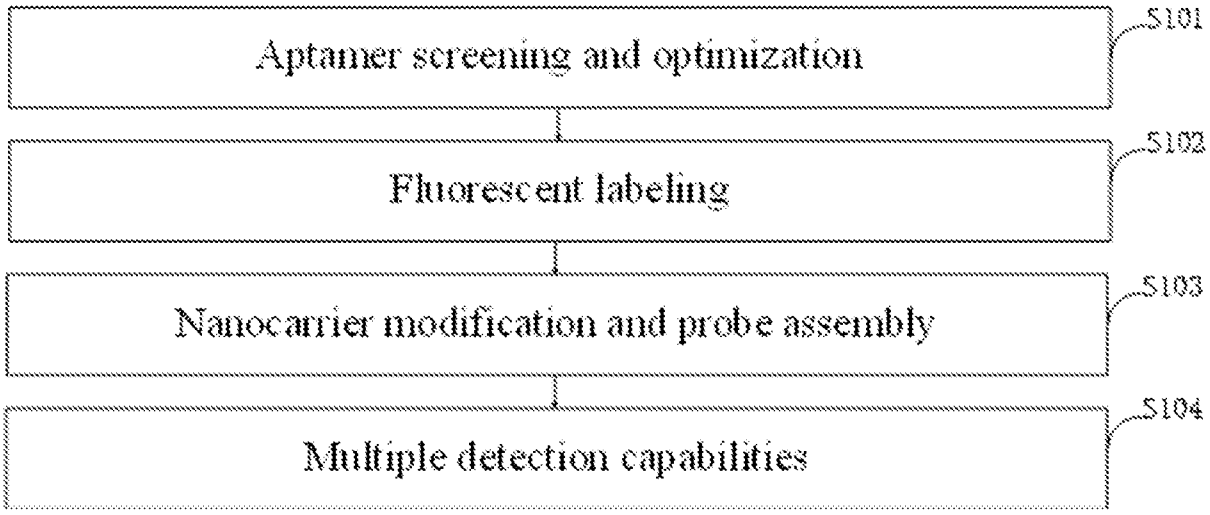

FIG. 1

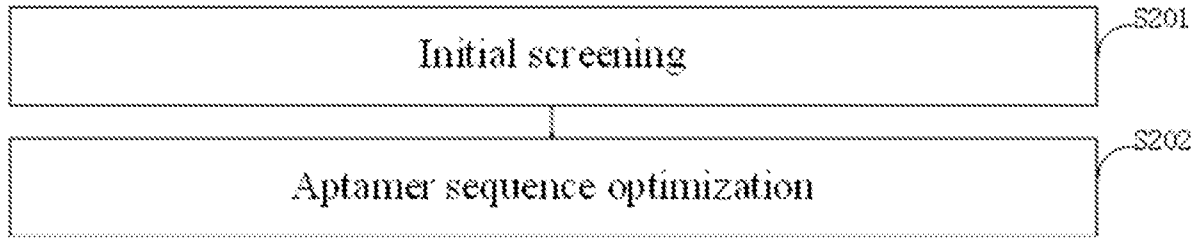

FIG. 2

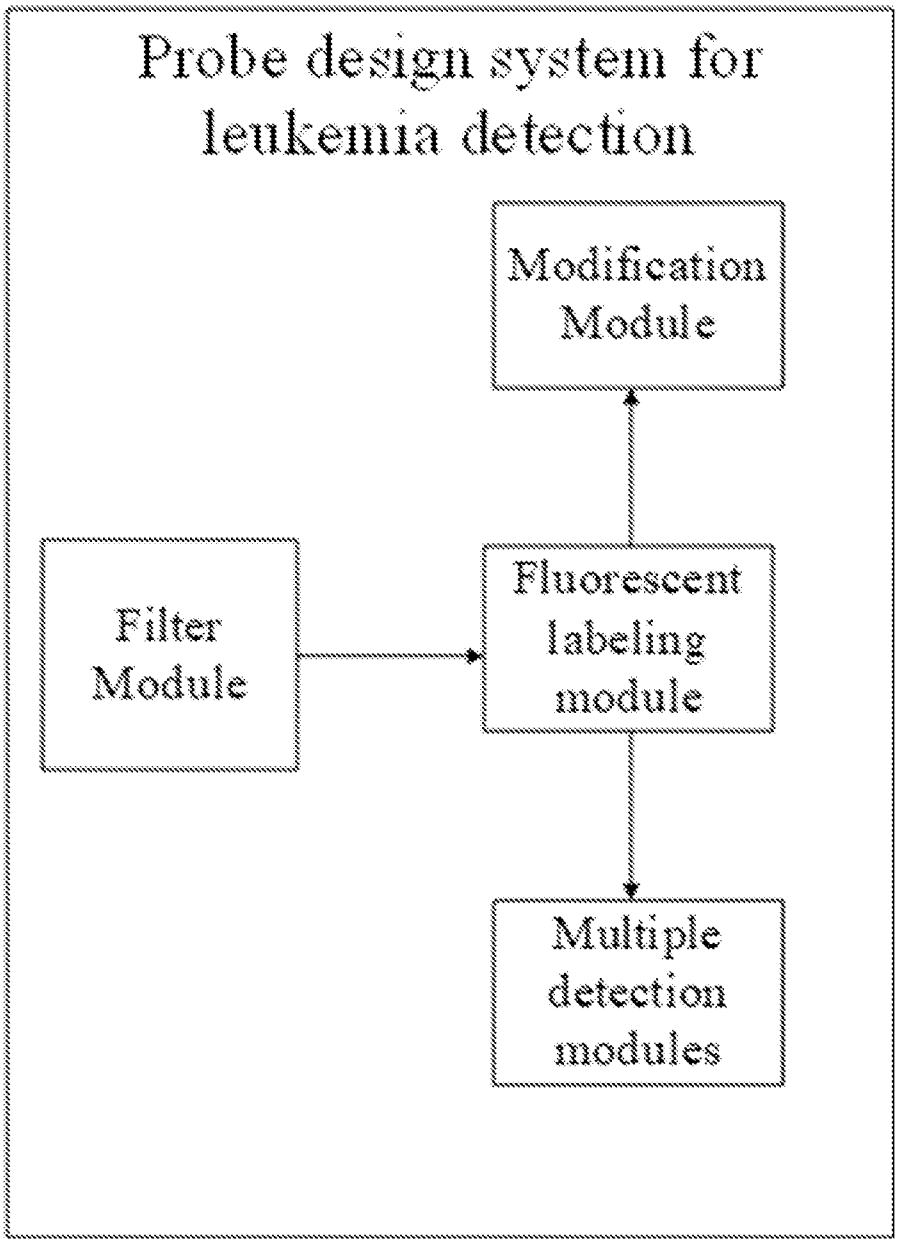
Probe design system for leukemia detection
Modification Module
Filter Module
Fluorescent labeling module
Multiple detection modules

FIG. 3