

CLAIMS

[Claim 1]

A DNA aptamer that binds to a cancer cell comprising the nucleotide sequence (i) or (ii) below:

(i) a nucleotide sequence represented by $N_1N_2N_3N_4N_5AGGGGTGTTTGTGCCGTGAGN_{26}TGTN_{30}N_{31}N_{32}N_{33}N_{34}$ (SEQ ID NO: 35), wherein N_1 to N_5 each independently represent G, T, A, or C, N_{26} represents 7-(2-thienyl)-3H-imidazo[4,5-b]pyridin-3-yl, N_{30} forms a base pair with N_5 , N_{31} forms a base pair with N_4 , N_{32} forms a base pair with N_3 , N_{33} forms a base pair with N_2 , and N_{34} forms a base pair with N_1 , and

(ii) a nucleotide sequence represented by $N_1N_2N_3N_4N_5N_6GATCACN_{13}N_{14}CTAGGGGN_{22}CCN_{25}N_{26}N_{27}N_{28}N_{29}N_{30}N_{31}$ (SEQ ID NO: 28), wherein, N_1 to N_6 , N_{14} , and N_{25} each independently represent G, T, A, or C, N_{13} and N_{22} each represent 7-(2-thienyl)-3H-imidazo[4,5-b]pyridin-3-yl, N_{26} forms a base pair with N_4 , N_{29} forms a base pair with N_3 , N_{30} forms a base pair with N_2 , N_{31} forms a base pair with N_1 , and N_{27} and N_{28} each independently represent G, T, A, or C or none).

[Claim 2]

The DNA aptamer according to claim 1, wherein the nucleotide sequence (i) or (ii) further comprises 1 to 5 GC base-pairs at its terminus.

[Claim 3]

The DNA aptamer according to claim 1 or 2, wherein the base pair is a GC pair.

[Claim 4]

The DNA aptamer according to claim 1 or 2, wherein the nucleotide sequence (i) is the sequence as shown in SEQ ID NO: 36, 37, or 40.

[Claim 5]

The DNA aptamer according to claim 1 or 2, wherein the nucleotide sequence (ii) is the sequence as shown in SEQ ID NO: 29.

[Claim 6]

The DNA aptamer according to any one of claims 1 to 5, which further comprises a mini-hairpin structure at the 3'-terminus, wherein the mini-hairpin structure is composed of the nucleic acid regions (A) to (C) below sequentially ligated from the 5'-terminus toward the 3'-terminus:

(A) a first nucleic acid region consisting of 2 to 5 random nucleotides;

(B) a second nucleic acid region consisting of a nucleotide sequence of GNA or GNNA (wherein each "N" independently represents any of G, T, A, or C); and

(C) a third nucleic acid region consisting of a nucleotide sequence complementary to the first nucleic acid region,

wherein the first nucleic acid region and the third nucleic acid region form a stem portion by base pairing, and the second nucleic acid region forms a loop portion.

[Claim 7]

A DNA aptamer that binds to a breast cancer cell comprising any of the nucleotide sequences (I) to (IV) below:

(I) a nucleotide sequence represented by $N_1N_2N_3N_4N_5N_6CAGCCTGGGN_{16}TN_{18}TCTTTN_{24}AGTCN_{29}AN_{31}TTCAN_{36}TCGN_{40}N_{41}N_{42}N_{43}N_{44}N_{45}$ (SEQ ID NO: 43), wherein N_1 to N_6 , N_{18} , N_{24} , N_{31} , and N_{36} each independently represent G, T, A, or C, N_{16} represents 7-(2-thienyl)-3H-imidazo[4,5-b]pyridin-3-yl, N_{29} represents 7-(2-thienyl)-3H-imidazo[4,5-b]pyridin-3-yl, G, T, A, or C, N_{40} forms a base pair with N_6 , N_{41} forms a base pair with N_5 , N_{42} forms a base pair with N_4 , N_{43} forms a base pair with N_3 , N_{44} forms a base pair with N_2 , and N_{45} forms a base pair with N_1 ;

(II) a nucleotide sequence represented by $N_1N_2N_3N_4CN_6N_7TCGAACTGN_{16}N_{17}ATGAGN_{23}GTN_{26}N_{27}N_{28}N_{29}N_{30}N_{31}$ (SEQ ID NO: 2), wherein N_1 to N_4 , N_7 , N_{16} , N_{23} , N_{26} , and N_{31} each independently represent G, T, A, or C, N_6 represents 7-(2-thienyl)-3H-imidazo[4,5-b]pyridin-3-yl, G, T, A, or C, N_{17} represents 7-(2-thienyl)-3H-imidazo[4,5-b]pyridin-3-yl, N_{27} forms a base pair with N_3 , N_{28} forms a base pair with N_2 , and N_{29} and N_{30} each independently represent G, T, A, or C or none;

(III) a nucleotide sequence represented by $N_1N_2N_3CCGGCTN_{10}N_{11}N_{12}N_{13}N_{14}N_{15}N_{16}N_{17}N_{18}N_{19}N_{20}N_{21}N_{22}N_{23}N_{24}N_{25}TCCTGGN_{32}N_{33}TAAGGTTTCTAAN_{46}N_{47}N_{48}N_{49}N_{50}N_{51}$ (SEQ ID NO: 18), wherein N_1 to N_3 , N_{13} to N_{17} , N_{19} to

N₂₂, N₃₃, N₄₆ to N₄₈, and N₅₀ each independently represent G, T, A, or C, N₁₀ to N₁₂ and N₂₃ to N₂₅ each independently represent G, T, A, or C or none, N₁₈ represents 7-(2-thienyl)-3H-imidazo[4,5-b]pyridin-3-yl, G, T, A, or C or none, N₃₂ represents 7-(2-thienyl)-3H-imidazo[4,5-b]pyridin-3-yl, N₄₉ forms a base pair with N₃, N₅₁ forms a base pair with N₁, and 3 or more of N₁₀ to N₁₅ form base pairs with 3 or more of N₂₀ to N₂₅; and

(IV) a nucleotide sequence represented by N₁N₂N₃TCTTAAGTTTAN₁₅AN₁₇N₁₈TN₂₀N₂₁N₂₂N₂₃TN₂₅N₂₆N₂₇N₂₈N₂₉N₃₀N₃₁N₃₂TN₃₄GGGCGTTTTAAN₄₆N₄₇N₄₈N₄₉ (SEQ ID NO: 50), wherein N₁ to N₃, N₁₅, N₁₇, N₂₀ to N₂₃, N₂₅ to N₂₉, N₃₁ to N₃₂, N₃₄, and N₄₆ each independently represent G, T, A, or C, N₁₈ and N₃₀ each represent 7-(2-thienyl)-3H-imidazo[4,5-b]pyridin-3-yl, N₄₇ forms a base pair with N₃, N₄₈ forms a base pair with N₂, and N₄₉ forms a base pair with N₁.

[Claim 8]

The DNA aptamer according to claim 7, wherein the nucleotide sequence of (I), (II), (III), or (IV) further comprises 1 to 5 GC base-pairs at its terminus.

[Claim 9]

The DNA aptamer according to claim 7 or 8, wherein the base pair is a GC pair.

[Claim 10]

The DNA aptamer according to claim 7 or 8, wherein the nucleotide sequence of (I) is the sequence as shown in SEQ ID NO: 44 or 46.

[Claim 11]

The DNA aptamer according to claim 7 or 8, wherein the nucleotide sequence of (II) is the sequence as shown in any of SEQ ID NOs: 3 to 5, 8 to 9, and 14 to 16.

[Claim 12]

The DNA aptamer according to claim 7 or 8, wherein the nucleotide sequence of (III) is the sequence as shown in any of SEQ ID NOs: 20, 21, and 26.

[Claim 13]

The DNA aptamer according to claim 7 or 8, wherein the nucleotide sequence of (IV) is the sequence as shown in SEQ ID NO: 51.

[Claim 14]

The DNA aptamer according to any one of claims 7 to 13, which further comprises a mini-hairpin structure at the 3'-terminus, wherein the mini-hairpin structure is composed of nucleic acid regions (A) to (C) below sequentially ligated from the 5'-terminus toward the 3'-terminus:

(A) a first nucleic acid region consisting of 2 to 5 arbitrary nucleotides;

(B) a second nucleic acid region consisting of a nucleotide sequence GNA or GNNA (wherein each "N" independently represents any of G, T, A, or C); and

(C) a third nucleic acid region consisting of a nucleotide sequence complementary to the first nucleic acid region,

wherein the first nucleic acid region and the third nucleic acid region form a stem portion by base pairing, and the second nucleic acid region forms a loop portion.

[Claim 15]

A cancer detecting agent comprising the DNA aptamer according to any one of claims 1 to 14.

[Claim 16]

A cancer cell detecting kit comprising the DNA aptamer according to any one of claims 1 to 14.

[Claim 17]

A pharmaceutical composition comprising the DNA aptamer according to any one of claims 1 to 14.

[Claim 18]

An anticancer agent consisting of the DNA aptamer comprising the nucleotide sequence (i) according to any one of claims 1 to 6.

[Claim 19]

A pharmaceutical composition for delivering a drug to a cancer cell, comprising the DNA aptamer according to any one of claims 1 to 14 and a drug.

[Claim 20]

A method for detecting a cancer cell comprising:

a step of contacting a sample containing cells obtained from a subject with the DNA aptamer according to any one of claims 1 to 6; and

a step of detecting a cancer cell based on the binding between the sample and the DNA aptamer.

[Claim 21]

A method for detecting a breast cancer cell comprising:

a step of contacting a sample containing cells obtained from a subject with the DNA aptamer according to any one of claims 7 to 14; and

a step of detecting a breast cancer cell based on the binding between the sample and the DNA aptamer.

[Claim 22]

A method for classifying cancer cells obtained from a subject comprising:

a step of contacting cancer cells obtained from a subject with the DNA aptamer according to any one of claims 1 to 14;

a step of determining the presence or absence of the binding between the cancer cells and the DNA aptamer, or measuring the strength of the binding; and

a step of classifying cancer cells based on the presence or absence of the binding, or the strength of the binding.

[Claim 23]

A method for classifying breast cancer cells obtained from a subject comprising:

a step of contacting breast cancer cells obtained from a subject with the DNA aptamer according to any one of claims 7 to 14;

a step of determining the presence or absence of the binding between the breast cancer cells and the DNA aptamer or measuring the strength of the binding; and

a step of classifying breast cancer cells based on the presence or absence of the binding, or the strength of the binding.

[Claim 24]

The method according to claim 23, wherein the step of contacting comprises contacting breast cancer cells obtained from a subject with two or more DNA aptamers selected from at least two groups among the four groups (I) to (IV) according to any one of claims 7 to 14.