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NUCLEIC ACID APTAMER, COMPOSITION, USE OF APTAMER, DIAGNOSTIC KIT, METHOD FOR DETECTING OR DIAGNOSING TUMOURS AND CANCER TREATMENT METHOD

Abstract

The present invention refers to nucleic acid aptamers comprising a nucleotides sequence with general formula (1), as follows, or a pharmaceutical acceptable salt thereof:
TAGGGAAGAGAAGGACATATGAT-X1-TTGACTAGTACATGACCAC TTGA (formula 1), wherein X1 is the nucleotides sequence as defined in any one of the SEQ ID NO: 1-10, SEQ ID NO: 21-30 e SEQ ID NO: 41-50, or a sequence with at least 90% identity to the same which presents a function equivalent to its corresponding. The present invention further refers to the composition comprising at least one aptamer as previously defined. The present invention further refers to the use of an aptamer as previously defined or a composition as previously defined for the manufacture of a drug for the treatment of cancer. The present invention further refers to the diagnostic kit comprising the aptamers as previously defined or the composition as previously defined. The present invention further refers to the method for detecting or diagnosing a tumor, which comprises contacting at least one aptamer as previously defined or a composition as previously defined with a cell, tissue, or sample from an individual, and detecting the binding of the aptamer to the cell, tissue, or sample. The present invention further refers to a method for treating cancer, comprising a step of administering to an individual a therapeutically effective amount of an aptamer as previously defined or of a composition as previously defined.

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Background/Summary**SEQUENCE LISTING**

[0001] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML file, created on Sep. 3, 2024, is named PE003408 Sequence Listing new.xml and is 55,928 bytes in size.

FIELD OF THE INVENTION

[0002] The present invention lies in the field of cellular and molecular biology. The present invention refers generally to nucleic acid aptamers, more particularly of DNA, for application in the diagnosis of prostate, ovarian and breast tumors. The present invention also refers to a composition, use of such aptamers or use of a composition, diagnostic kit, method for detecting or diagnosing a tumor and method for treating cancer.

BACKGROUNDS OF THE INVENTION

[0003] Cancer is the second major cause of death in the world, responsible for more than 9 million deaths a year. Around 60% of cancer cases are diagnosed at an advanced stage and, as a result of late diagnosis, in addition to a lower chance of curing the patient, the cost of therapies can rise by up to 80%. Investing in early diagnosis means reducing the impact of cancer on the population and the public budget.

[0004] Many types of cancer, of high relevance to public health, can be cured if detected correctly at an early stage and treated appropriately (Goldie and Daniels, 2011). Treatment for cancer is still based on invasive methods with limited specificity such as chemotherapy, radiotherapy, and surgery. The major disadvantage of conventional therapies is the lack of specificity of their mode of action, which can cause serious side effects by killing normal, non-cancerous cells. The limited efficacy of drugs is currently one of the main barriers to cancer treatment, mainly due to insolubility, systemic toxicity, and drug resistance, made worse by debilitating side effects. Thus, the search for promising diagnostic strategies, more accurate prognosis and new therapeutic approaches are crucial for effective treatment with minimal side effects. Monoclonal antibodies are increasingly being used to treat cancer (Rosenberg and Dudley, 2009).

[0005] Currently, studies are focused on therapies with specific targets for cancer cells that do not damage healthy cells. New strategies and tools for treatment, such as the use of recombinant Fab antibodies, or fusions between the variable regions of heavy and light chains ("single chain variable fragments"—scFv) or multifunctional dia- and triabodies and aptamers, among several others, are under development and validation, and can be called nanomolecules that help contribute directly to

knowledge about the biology of tumors such as heterogeneity, both inter- and intra-tumoral for potential personalized treatment (Hanahan, 2014, Rashid, 2017).

[0006] Nanomedicine is a promising field in cancer treatment. Currently, several nanoscale compound carriers are undergoing clinical screening (Phases I to III, depending on the study) and formulations are being developed using nanoformulated polymers containing RNA interference or associated with compounds such as docetaxel, paclitaxel and cisplatin, all of which have therapeutic potential for solid tumors of the prostate, breast and kidney, respectively. Through nanomedicine it is possible to use different strategies for diagnosis, prognosis, and new therapeutic approaches, supported by real-time monitoring of pharmacokinetics, therapeutic accumulation, and disease progression (Bourzac, 2012).

[0007] In tumor cells, the levels of some cell surface markers can increase, decrease, undergo modifications, or new markers can emerge. These molecular variations can be used to differentiate healthy cells from tumor cells (Germer and Mann, 2013; Kampen, 2011). Many oncogenic processes take place primarily at the plasma membrane, including proliferation, adhesion, and migration (Ziegler et al, 2014). Thus, proteins differentially expressed in tumors have great clinical relevance and can be the molecular target of structures with high affinity and specificity, such as monoclonal antibodies or fragments thereof, and aptamers, which may be able to directly or indirectly induce the death of tumor cells, inhibit disease progression, be a specific tumor biomarker, representing an important tool for tumor diagnosis, being able to distinguish tumor cells from normal ones (Shi et al., 2017).

[0008] Therefore, aptamers represent an important tool that can be used to improve therapeutic specificity and differential diagnosis of cancer.

[0009] Aptamers are small oligonucleotides of 60-100 bases, which can be DNA or RNA, assuming unique three-dimensional (3D) structures, with the potential to bind with high affinity and specificity to molecular targets (Chen et al., 2017).

[0010] SELEX consists of an in vitro selection method for aptamers of interest by exponential enrichment, which enriches aptamers bound with high affinity to target molecules (Tuerk and Gold, 1990; Ellington and Szostak, 1990). The first step is, from a library of chemically synthesized oligonucleotides (between 10¹³ and 10¹⁶ oligos of different sequences): (1) conducting the binding, by incubating the library of oligonucleotides with the selected target; (2) separating and isolating sequences bound to the target from those that were not bound; (3) eluting the complex by chromatography; (4) amplifying, generating a new nucleic acids pool by PCR (DNA) or RT-PCR (RNA); (5) in vitro conditioning all transcribed and purified relevant ssDNA. While SELEX uses isolated molecules, Cell SELEX uses entire cells as a target (Sefah et al., 2010).

[0011] Cell SELEX is a method for selecting aptamers in whole cells, especially for detecting surface proteins that are overexpressed in tumor cells (Coulter et al., 1997; Sefah et al., 2010; Ohuchi, 2012). The use of Cell SELEX has advantages, as it is based on the selection of aptamers against molecules on the surface of living cells, which maintain their native conformations and thus preserve their biological functions.

[0012] Wu et al. review the applications of DNA and RNA aptamers in the area of cancer theranostics. This document mentions diagnostic methods already developed that make use of aptamers through optical and electrochemical aptasensors, among others. More specifically in therapeutic methods aimed at cancer, the AS 1411 aptamers (under Phase II clinical development for the treatment of leukemia) and the SGC8 Doxorubicine (DOX) developed by Aptamera (Louisville, KY) are highlighted (WU et al., 2015).

[0013] Blank et al., 2001 applies the Cell SELEX system on a library comprising 10^{sup}.15 single-stranded DNA aptamer sequences. From 25 aptamers analyzed, one aptamer was selected (III.1) by selectively binding to tumor cell proteins without binding to non-tumor cells, including cells close to tumor cells. More specifically, aptamer 111.1 is an ssDNA ligand (96 bases) that targets endothelial proteins (YPEN-1) from the Ewing sarcoma family. The action of this aptamer

prevented the endothelial cell proliferation process, a key process in angiogenesis.

[0014] Jayasena, S. D. points out several technologies with aptamers. Several applications in diagnostics have been described, such as double-site binding or “sandwich” analysis, flow cytometry, sensors, and fluorescence polarization. In conclusion, the review predicts that the SELEX strategy is useful for identifying aptamers with targets in complex mixtures, such as searching for aptamers to bind to an epitope present exclusively on the surface of tumor cells, but not on normal cells, aiding in the diagnosis of different types of cancer (p. 1631, § 5). This document, although outdated (1999), is one of the first to point out the combination of the SELEX methodology in obtaining aptamers for cancer.

[0015] U.S. Pat. No. 10,274,497 B2 describes ovarian cancer-specific aptamer and ovarian cancer detection method. D3 has an ssDNA library synthesized by Medclub scientific Co. (Taiwan), with 72 bases. Specifically, each ssDNA includes a region of 40 random bases and 2 constant regions of 16 bases each. The aptamers are then selected by Cell SELEX after incubation with OVCAR-3 cells. The detection method consists of providing at least one of the aptamers (SEQ ID NO: 2 and/or 3—hereinafter referred to as Aptamer A and B); mixing the sample with an aptamer to allow binding; and detecting the aptamer that has bound (p. 2, lines 20-34). Furthermore, the SELEX method is combined with an integrated microfluidics system to select aptamers A and B, with specificity for the OVCAR-3 ovarian cancer line, is described. (page 3, lines 10-18).

[0016] U.S. Pat. No. 9,790,507 B2 describes an aptamer with a defined sequence whose composition can be used in the treatment and diagnosis of cancer, in particular solid tumors. The method for selection is Cell SELEX, and the cell line used was human mammary gland adenocarcinoma (MCF-7). The document describes the selection of aptamers for cell line adenocarcinomas (Example 1—Selection of aptamers). Said aptamer with a defined biological sequence is DNA 5'-GCTGTGTGACTCCTGCAA-N43-GCAGCTGTATCTTGTCTCC-3' (wherein N43 is chosen from a group of nucleotides, SEQ ID NO: 2-13, with fixed length of 43 bases—see page 5).

[0017] U.S. Pat. No. 9,783,808 B2 discloses an aptamer with SEQ ID NO: 1, a composition comprising the same, diagnosis and therapy for cancer with the mentioned aptamer (Abstract). An in vitro selection method using SELEX and a microfluidic chip system is described. 13 aptamers are identified—FIG. 2(A) to 2(K)—that can bind to different types of ovarian cancer cells.

[0018] KR20180094764 describes aptamers selected in tumor cell lines (OVCAR-3) that specifically bind to the CA125 tumor antigen, a known biomarker for ovarian cancer. The objective is to obtain aptamers that recognize highly specific biomarkers for ovarian cancer.

[0019] US2019317099 describes aptamers selected for microvesicle surface antigens, using the SELEX method with HIS-tag on Dynabeads, wherein DNA and RNA aptamers, the DNA/RNA mix and a pool of aptamers are considered, with said method being capable of detecting any type of tumor. It turns out that the aforementioned document does not allow the detection of selected aptamers for live tumor cells, only for microvesicles. Another noteworthy point refers to the fact that document US2019317099 does not use the Cell SELEX method, a method capable of enabling the possibility of new markers, selecting, and identifying specific aptamers for each tumor type, allowing specific tumor identification, the ability to differentiate between tumors and selective therapy.

[0020] Today, the diagnostic methods used to detect prostate and ovarian tumors are inconclusive as they are non-specific, based on serological biomarkers (PSA and CA125, respectively). Digital rectal exams and ultrasound imaging are complementary, but still inconclusive, especially in the case of ovarian tumors. Thus, in most suspected cases of ovarian cancer, the diagnosis is only completed after exploratory surgery, which can last many hours, which makes the process expensive, time-consuming and, above all, very painful for the patient.

[0021] Still in what regards the diagnosis of ovarian tumor, transvaginal ultrasound is usually used to assess the presence of a tumor mass in the ovaries and the CA-125 protein dosage test in

peripheral blood, which is present at increased levels in several cases. Despite this achievement, there are no specific biomarkers for therapy and early diagnosis, despite having the highest mortality rate among all gynecological tumors (Grunewald and Ledermann, 2017). Therefore, obtaining a set of tumor-specific aptamers (more specifically OVCAR-3), as presented in the present invention, has great potential for application and use in therapy, diagnosis or as a drug carrier for patients with ovarian cancer, with minimal side effects on the healthy cells.

[0022] In the case of breast cancer, although the molecular markers are very specific for tumor classification, the triple negative subtype does not show expression of the receptors used as classifiers and is therefore diagnosed by exclusion, without presenting a specific marker.

Furthermore, triple negative breast cancer, which is diagnosed in approximately 15% of women, is characterized by the subtype with greater invasive potential and worse prognosis. Thus, among other embodiments, the present invention proposes diagnostic kits based on aptamers specific for the detection of prostate, ovarian and breast tumor cells. These kits can be used as a non-invasive method, applied to liquid biopsy, and in positive cases, they can be used in magnetic resonance imaging to detect a tissue tumor area, thus avoiding invasive and high-cost methods such as exploratory surgery, today often used to conclusion of the diagnosis.

[0023] Additionally, the invention provides a non-invasive, specific, effective, and affordable early detection method, which can also differentiate the metastatic potential of circulating tumor cells.

[0024] The invention will be presented in greater detail below.

SUMMARY OF THE INVENTION

[0025] In a first aspect, the invention provides a nucleic acid aptamer which comprises a nucleotides sequence with general formula (1), as follows, or a pharmaceutical acceptable salt thereof: TAGGGAAGAGAAGGACATATGAT-X1-TTGACTAGTACATGACCACTTGA (formula 1), wherein X1 is the nucleotides sequence as defined in any one of the SEQ ID NO: 1-10, SEQ ID NO: 21-30 and SEQ ID NO: 41-50, or a sequence with at least 90% identity to the same. In one embodiment, the nucleic acid is DNA. In another embodiment, the aptamer is for use as a drug or a diagnostic reagent. In another embodiment, the aptamer is for use in the diagnosis and treatment of cancer. In another embodiment, the cancer is selected from the group consisting of prostate, ovarian and breast cancer. In another embodiment, the breast cancer is breast cancer of the triple negative subtype.

[0026] In a second aspect, the invention provides a composition which comprises at least one aptamer as defined above. In one embodiment, the composition further comprises one or more pharmaceutically acceptable carriers, excipients, or solvents. In another embodiment, the composition additionally comprises one or more additional active ingredients. In another embodiment, the composition is for use in the detection, diagnosis, or treatment of cancer. In another embodiment, the cancer is selected from the group consisting of prostate, ovarian and breast cancer. In another embodiment, the breast cancer is the triple negative breast cancer.

[0027] In a third aspect, the invention provides the use of an aptamer as defined above or of a composition as defined above for the manufacture of a drug for the treatment of cancer. In one embodiment, the cancer is selected from the group consisting of prostate, ovarian and breast cancer. In another embodiment, the breast cancer is the triple negative subtype.

[0028] In a fourth aspect, the invention provides a diagnostic kit which comprises the aptamers as defined above, or the composition as defined above.

[0029] In a fifth aspect, the invention provides a method for detecting or diagnosing a tumor, which comprises contacting at least one aptamer as defined above or a composition as defined above with a cell, tissue, or sample from an individual, and detecting the binding of the aptamer to the cell, tissue, or sample. In one embodiment, the aptamer is associated with a fluorophore, an image contrast agent or a radioisotope. In another embodiment, the cell, tissue, or sample is of prostate, ovarian and breast cancer.

[0030] In a sixth aspect, the invention provides method for treating cancer, which comprises a step of

administering to an individual a therapeutically effective amount of an aptamer as defined above or of a composition as defined above.

Description

BRIEF DESCRIPTION OF THE FIGURES

[0031] The objective of the invention, together with additional advantages thereof, can be better understood by referring to the attached figures and the following descriptions:

[0032] FIG. 1. FIG. 1 is the schematic representation of the structure of aptamers in the library.

[0033] FIG. 2. FIG. 2 is the schematic representation of the Cell SELEX.

[0034] FIG. 3. FIG. 3 is the schematic representation of the Next Generation Sequencing method (Fusion PCR Method—ION PGM™) to identify the aptamer sequences obtained by Cell SELEX. The adapter sequence A together with the key sequence and the Barcode are produced and supplied commercially and determine the signature of each set of aptamers selected by Cell SELEX. The adapter sequence P1, also supplied commercially, is present in all aptamer sequences. First, the reaction to bind the adapters to the aptamers is conducted and then an emulsion PCR, with primers complementary to adapters A and P1, is conducted to exponentially amplify these sequences.

[0035] FIG. 4. FIG. 4 shows N3DBioprinting®. (A)—Product presentation; (B)—Base with the magnetic buttons; (C)—3D spheroid growing plate; and (D)—Spheroid formation (“printing”).

Source: www.n3dbio.com

[0036] FIG. 5. FIG. 5 shows microscopy images of tumor and non-tumor control cells grown in specific medium for each cell type: A—LNCaP; B—RWPE-1; C—OVCAR-3; D—CHO-K1; E—MDA-MB-231; and F—MCF 10A. The cultures were kept in an incubator at 37° C. in a 5% CO₂ atmosphere and used for the Cell SELEX assays. Morphological characteristics of the cells in monolayer, viewed under a light field microscope, magnification 10×.

[0037] FIG. 6. FIG. 6 shows 3% agarose gel for verification of aptamer pool amplification in the 12th round of Cell SELEX after incubation with prostate tumor cell line (LNCaP). A—Better cycling verification PCR with Cell SELEX performed at 37° C.—channels 1 to 5—and Cell SELEX performed at 4° C.—channels 6 to 10. Channels 1 and 6 correspond to 4 PCR cycles; channels 2 and 7, 6 PCR cycles; channels 3 and 8, 8 PCR cycles; channel 4 and, 10 PCR cycles/channels 5 and 10, 12 PCR cycles. B—Preparatory PCR (Primer Forward) conducted with the number of cycles chosen in the best cycle verification PCR (10 cycles) in Cell SELEX at 37° C. (channel 11) and 4° C. (channel 12).

[0038] FIG. 7. FIG. 7 shows the evaluation of dose-response linkage with the prostate tumor line, LNCaP, incubated with aptamers selected by Cell SELEX after the 12th round for 1 h at 37° C., in concentrations of 5, 10 e 20 μM of aptamers. A—Cells incubated with the aptamers library; B—Cells incubated solely with the primer labeled with FITC; C—Cells incubated with 12R ssDNA aptamers at 5 μM; D—Cells incubated with 12R ssDNA aptamers at 10 μM; E—Cells incubated with 12R ssDNA aptamers at 20 μM.

[0039] FIG. 8. FIG. 8 shows the cytometry assay for evaluating the enrichment of the ssDNA aptamer pool in tumor and non-tumor control cells, LNCaP and RWPE-1 respectively, between the 6th and 12th round of Cell SELEX. (A) Histogram representing the average fluorescence intensity of LNCaP treated with FITC aptamers from the 6th to the 12th round; (B) Graphical representation of the percentage of the number of fluorescent LNCaP cells (incubated with FITC aptamers) within the selected population (R1); (C) Graphical representation of the average fluorescence intensity of LNCaP cells within the selected population (R1); (D) Histogram representing the average fluorescence intensity of LNCaP and RWPE-1 treated with FITC aptamers in the 12th round within the selected population (R1); (E) Graphical representation of the percentage of the number of LNCaP and RWPE-1 cells treated with FITC aptamers in the 12th round within the selected

population (R1).

[0040] FIG. 9. FIG. 9 shows 3% agarose gel for verification of aptamer pool amplification in the 12th round of Cell SELEX after incubation with breast tumor line (MDA-MB-231). A—PCR to verify best cycling with Cell SELEX conducted at 37° C.—channels 1 to 4—channel number 1 corresponds to 6 PCR cycles; channel 2, 8 PCR cycles; channel 3, 10 PCR cycles; channel 4, 12 PCR cycles. B—Preparatory PCR (Primer Forward) conducted with the number of cycles chosen in the best cycle verification PCR (10 cycles) in Cell SELEX conducted at 37° C. (channel 5).

[0041] FIG. 10. FIG. 10 shows the cytometry assay for evaluating the enrichment of the ssDNA aptamer pool in tumor and non-tumor control cells, MDA-MB-231 and MCF-10A respectively, between the 6th and 12th round of Cell SELEX. (A) Histogram representing the average fluorescence intensity of MDA-MB-231 treated with FITC aptamers from the 6th to the 12th round; (B) Graphical representation of the percentage of the number of fluorescent MDA-MB-231 cells (treated with FITC aptamers) within the selected population (R1); (C) Graphical representation of the average fluorescence intensity of MDA-MB-231 cells within the selected population (R1); (D) Histogram representing the average fluorescence intensity of MDA-MB-231 and MCF-10A treated with FITC aptamers in the 12th round within the selected population (R1); (E) Graphical representation of the percentage of the number of MDA-MB-231 and MCF-10A cells treated with FITC aptamers in the 12th round within the selected population (R1).

[0042] FIG. 11. FIG. 11 shows 3% agarose gel for verification of aptamer pool amplification in the 16th round of Cell SELEX after incubation with ovarian tumor cell line (OVCAR-3). A—PCR to verify best cycling with Cell SELEX conducted at 37° C.—channels 1 to 4—channel number 1 corresponds to 6 PCR cycles; channel 2, 8 PCR cycles; channel 3, 10 PCR cycles; channel 4, 12 PCR cycles. B—Preparatory PCR (Primer Forward) conducted with the number of cycles chosen in the best cycle verification PCR (12 cycles) in Cell SELEX conducted at 37° C. (channel 5).

[0043] FIG. 12. FIG. 12 shows the cytometry assay for evaluating the enrichment of the ssDNA aptamer pool in tumor and non-tumor control cells, OVCAR-3 and CHO-K1 respectively, between the 6th and 16th round of Cell SELEX. (A) Histogram representing the mean fluorescence intensity of OVCAR-3 treated with FITC aptamers from the 6th to the 16th round; (B) Graphical representation of the percentage of the number of fluorescent OVCAR-3 cells (incubated with FITC aptamers) within the selected population (R1); (C) Graphical representation of the mean fluorescence intensity of OVCAR-3 cells within the selected population (R1); (D) Histogram representing the average fluorescence intensity of OVCAR-3 and CHO-K1 treated with FITC aptamers in the 16th round within the selected population (R1); (E) Graphical representation of the percentage of the number of OVCAR-3 and CHO-K1 cells treated with FITC aptamers in the 16th round within the selected population (R1).

[0044] FIG. 13. FIG. 13 shows the 30 to 240 minute kinetics assay for evaluating the binding profile of selected 12R ssDNA aptamers against prostate tumor cells (LNCaP) at increasing concentrations (1 μ M to 100 μ M), performed on the high content analysis (HCS) equipment (10 \times magnification).

[0045] FIG. 14. FIG. 14 shows the morphological profile of LNCaP prostate tumor cells when incubated with 12R ssDNA aptamers labeled with FITC at 1 μ M for 30 to 240 minutes.

Highlighted (arrows) is the change in cell profile.

[0046] FIG. 15. FIG. 15 shows the dose-dependent evaluation of cell morphology under an optical microscope (20 \times) of prostate tumor cells incubated with aptamers-FITC at concentrations of 0.25 to 50 μ M for 24 hours.

[0047] FIG. 16. FIG. 16 shows the analysis of cell viability through the Prestoblu[®] assay in tumor cells and prostate control incubated with 12R ssDNA aptamers for 24 hours in increasing concentrations (0.25 to 50 μ M). A—Tumor cells (LNCaP); B—Non-tumor control cells (RWPE-1). Triton X-100 was used as a positive control to induce loss of cell viability. The aptamer library (“library”, total initial aptamers pool used in Cell SELEX), was used as an internal control to check

cell viability.

[0048] FIG. 17. FIG. 17 shows the analysis of cell proliferation using the Cyquant® assay (Thermofisher®) in prostate tumor and control cells incubated with 12R ssDNA aptamers for 24 hours at increasing concentrations (0.25 to 100 μ M). A—Tumor cells (LNCaP); B—Non-tumor control cells (RWPE-1). Triton X-100 was used as a positive control to induce loss of cell viability and inhibit cell proliferation.

[0049] FIG. 18. FIG. 18 shows the qualitative analysis of the induction of cell death by apoptosis using the TUNEL® technique (Terminal deoxynucleotidyl transferase Uracil Nick end Labeling) in prostate tumor cells incubated with 12R aptamers for 24 hours at increasing concentrations of 25 μ M and 50 μ M. The control is the LNCaP cell without aptamer treatment. The cell nucleus labeled with DAPI (1: 10,000) is represented by (1) and the fragmented DNA labeled with FTIC is represented by (2).

[0050] FIG. 19. FIG. 19 shows the Western Blot assay to investigate caspase pathway activation after incubation of tumor and non-tumor control cells, LNCaP and RWPE-1 respectively, with 25 μ M of ssDNA aptamers for 3 hours. Total protein extracts from LNCaP cells treated with ssDNA aptamers (+), LNCaP cells not treated with ssDNA aptamers (–), RWPE-1 cells treated with ssDNA aptamers (+) and, RWPE-1 cells not treated with ssDNA aptamers (–). The Western Blot analyses and the histogram to verify the expression levels of Caspase 10 (A), Caspase 9 (B) and Bax (C) were normalized in relation to the levels of GAPDH (endogenous control). Molecular mass standard used: PageRuler Plus Prestained Protein Ladder (Thermo Scientific®) (*P<0.05).

[0051] FIG. 20. FIG. 20 shows the ELISA assay to evaluate TRAIL expression in LNCaP and RWPE-1 cells incubated with 12R ssDNA aptamers for 3 hours.

[0052] FIG. 21. FIG. 21 shows the pool of 12R ssDNA aptamers that induce caspase 3 activation in prostate tumor cells, LNCaP. Detection of caspase 3 activity using the live cell assay NucView® 488 and CF®594 Annexin V Dual Apoptosis Assay Kit. Marking of activated caspase 3 (3) in LNCaP cells incubated with 25 μ M of 12R ssDNA aptamers at the indicated times (20-180 minutes with a 20-minute interval). Annexin V staining (4) to visualize phosphatidylserine exposure; and calcein (5) to observe viable cells. In J, K and L magnified field of the images in A, B and C, respectively.

[0053] FIG. 22. FIG. 22 shows the quantification of caspase 3 activity detection using the NucView® 488 and CF®594 Annexin V Dual Apoptosis Assay Kit in live cells. Marking of activated caspase 3 in LNCaP (A) and RWPE-1 (B) cells incubated with 25 μ M of 12R ssDNA aptamers at the indicated times (20-180 minutes with a 20-minute interval), represented in the dark gray line; cells without induction were incubated only with binding buffer, represented by the medium gray color; and cells treated with dexamethasone at 1 μ M are represented in the light gray color.

[0054] FIG. 23. FIG. 23 shows the assay to evaluate the formation capacity of 3D microspheres in ovarian tumor cells (OVCAR-3) and the non-tumor control (CHO-K1) using the “n3D System technology”, developed by Nano3D Biosciences, Inc®. Different amounts of OVCAR-3 and CHO-K1 (25×10^{sup.3}, 50×10^{sup.3} and 75×10^{sup.3}) were incubated with the magnetized nanoparticles in a 96-well magnetic plate (Bioprinting) for two hours. Once the spheroids had formed, the base was removed, and the culture was maintained in the specific medium for each cell type (10× magnification).

[0055] FIG. 24. FIG. 24 shows the assay to evaluate the formation capacity of 3D microspheres in prostate tumor cells (LNCaP) and the non-tumor control (RWPE-1) using “n3D System technology”, developed by Nano3D Biosciences, Inc®. 50×10^{sup.3} cells were incubated with the magnetized nanoparticles in a 96-well magnetic plate (Bioprinting) for two hours. Once the spheroids had formed, the base was removed, and the culture was maintained in the specific medium for each cell type (10× magnification).

[0056] FIG. 25. FIG. 25 shows the evaluation of microsphere cultures of tumor and control ovary

cells (OVCAR-3 and CHO-K1, respectively) incubated with a pool of 16R ssDNA aptamers for 24 hours. After 24 hours of cultivation of the spheroids (FIG. 23), 25 and 50 μ M of 16R ssDNA aptamers were added to the cultures and maintained for 24 hours. After 24 hours of incubation with the ssDNA aptamers, the spheroids were fixed with 4% PFA and marked with DAPI to visualize the volume of the microsphere (n=1).

[0057] FIG. 26. FIG. 26 shows the evaluation of microsphere cultures of tumor and prostate control cells (LNCaP and RWPE-1, respectively) incubated with a pool of 12R ssDNA aptamers for 24 hours. After 24 hours of cultivation of the spheroids (FIG. 24), 25 μ M of 12R ssDNA aptamers were added to the cultures and maintained for 24 hours. After 24 hours of incubation with the ssDNA aptamers, the spheroids were fixed with 4% PFA and marked with DAPI to visualize the volume of the microsphere (n=1).

[0058] FIG. 27. FIG. 27 shows the evaluation of the effect of the pool of ssDNA aptamers on 3D cultures of ovarian and prostate tumor cells (OVCAR-3 and LNCaP, respectively). After 24 hours of incubation of the spheroids with the 25 μ M of ssDNA aptamers, the spheroids of the two lines (OVCAR-3 and LNCaP) were labeled with DAPI, LysoTracker-Red and Phalloidin-FITC to visualize the nucleus, lysosomes, and cytoskeleton, respectively.

DETAILED DESCRIPTION OF THE INVENTION

[0059] Unless defined differently, all technical and scientific terms used herein have the same meaning as understood by a person skilled in the art to which the invention belongs. Conventional techniques of molecular biology and immunology are well known to a person skilled in the art. The specification also provides definitions of terms to assist in the interpretation of what is described herein and the claims. Unless otherwise indicated, all numbers expressing amounts, percentages and proportions, and other numerical values used in the specification and claims, should be understood as being modified, in all cases, by the term “about”. Therefore, unless otherwise indicated, the numerical parameters shown in the specification and in the claims are approximations that may vary, depending on the properties to be obtained.

Definitions

[0060] The term “aptamers”, in the context of the present invention, refers to nucleic acid sequences that adopt a specific 3D structure that allows them to bind to molecular targets with high specificity and affinity.

[0061] The term “nucleic acid”, according to the present invention, refers to any type of nucleic acid, such as DNA and RNA, and variants thereof, as well as combinations thereof, modifications thereof, including modified nucleotides etc. The terms “nucleic acid”, “oligonucleotide” and “polynucleotide” are used interchangeably in the context of the present invention. Nucleic acids can be purified from natural sources, produced using recombinant expression systems, and optionally purified, chemically synthesized, etc. Where appropriate, for example in the case of chemically synthesized molecules, the nucleic acids may comprise nucleoside analogues such as analogues having chemically modified bases or sugars, backbone modifications, etc. A nucleic acid sequence is represented in the 5'-3' direction unless otherwise indicated. As used herein, symbols for nucleotides and polynucleotides are those recommended by the IUPAC-IUB Commission of Biochemical Nomenclature (Biochem. 9: 4022, 1970).

[0062] As used in the present invention, the use of the term “pharmaceutically acceptable” essentially means not being toxic to the individual to whom the pharmaceutically acceptable material is administered.

[0063] The term “functionally equivalent variant” refers to the aptamers with sequences substantially similar to those SEQ ID NOs: 1-10, 21-30 or 41-50 maintaining the ability to specifically bind and/or inhibit its target. A functionally equivalent variant of the aptamer of the invention can be a nucleic acid sequence derived from SEQ ID NOs: 1-10, 21-30 or 31-40 which comprises the addition, substitution, or modification of one or more nucleotides. By way of illustration, functionally equivalent variants of the aptamer of the invention include sequences

which comprise the addition, removal, substitution or modification of 1 to 3 nucleotides of the sequences SEQ ID NOs: 1-10, 21-30 or 41-50 and which maintain an ability to specifically bind to their target and even inhibit it.

[0064] More specifically, the term “identity” is defined as the degree of equality between DNA or RNA sequences when compared nucleotide by nucleotide with the reference sequence.

[0065] In the present invention, the term “sequence identity percentage” refers to comparisons between polynucleotides and is determined by the sequences ideally aligned, under certain comparison parameters. This alignment may include gaps, generating gaps when compared to the reference sequence, which facilitates an adequate comparison of them. In general, the calculation of the identity percentage considers the number of positions where the same nucleotide occurs in the sequences compared to the reference sequence, and is conducted using various sequence comparison algorithms and programs known in the state of the art. Such algorithms and programs include, but are not limited to, BLAST and CLUSTAL, for example.

[0066] The present invention also includes aptamers which comprise nucleotides sequences with a sequence identity of at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% at least 98% or at least 99% with the sequences SEQ ID NOs: 1-10, 21-30 or 41-50 which, together with the sequences of the repeating ends, maintain an ability to specifically bind to their target and inhibit it.

[0067] Throughout this specification, unless the context otherwise requires, the word “comprise”, or variations such as “comprises” or “comprising”, will be understood as implying the inclusion of an indicated step or element or integer or group of elements or steps or integers but not to the exclusion of any other step or element or integer or group of elements or integers.

[0068] The term “consists of” or “consisting of” should be understood in the sense that a method, process, or composition of matter has the steps and/or components mentioned and no additional steps or components.

[0069] In the present application, the diagnostic reagent refers to the aptamer used to monitor the presence and/or progression of disease and response to therapy.

[0070] The terms “treat”, “treating” and “treatment” refer to a method of alleviating or nullifying a disease and/or its concomitant symptoms.

[0071] The term “cancer” refers to an abnormal, autonomous, and uncontrolled proliferation of cells from a specific tissue in the body. The term “cancer” can refer, for example, to lymphomas, multiple myelomas, hematological malignancies, leukemias, neoplasms and solid tumors and their metastases. In a preferred embodiment, the cancers are prostate, breast and ovarian.

[0072] According to the present invention, “carriers”, “excipients” or “pharmaceutically acceptable solvent” seeks to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like compatible with pharmaceutical administration. The use of such carriers and vehicles in pharmaceutically active substances is well known in the art. Unless any conventional carrier is incompatible with the active compound, its use in the compositions of the invention is contemplated. Acceptable vehicles, excipients or stabilizers are non-toxic to the individual at the doses and concentrations used and include buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzethonium chloride; phenolic, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol and m-cresol); low molecular weight polypeptides (less than about 10 amino acids); proteins, such as serum albumin, gelatine or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine or lysine; monosaccharides, disaccharides and other carbohydrates including glucose or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counterions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-

ionic surfactants such as TWEEN™, PLURONIC™ or polyethylene glycol (PEG). In one embodiment, the preferred vehicle is polyethylene glycol (PEG).

[0073] According to the present invention, the “active ingredients” may also be incorporated into the composition. Therefore, in one particular embodiment, the composition provided by the present invention may also contain more than one active ingredient as required for the particular indication where they are, preferably, those with complementary activities that do not adversely affect each other. For example, it may be desirable to provide, in addition, a chemotherapeutic agent, a cytokine, an analgesic agent, an anti-inflammatory agent or an immunosuppressive agent. The effective amount of these other active ingredients depends, among other things, on the therapeutic amount of aptamers present in the composition, the nature and severity of the pathology to be treated, the individual, etc.

[0074] According to the present invention, “fluorophores” refers to a functional component of a molecule that can make another molecule conjugate to it fluorescent by absorbing energy of a specific wavelength, emitting energy at different wavelengths. Among the possible fluorophores used in the present invention we can mention Fluorescein Isothiocyanate (FITC), Propidium Iodide, Rhodamine, Tetramethylrhodamine (TRITC) and other Rhodamine derivatives, Alexa Fluor 594, Texas Red, Alexa Fluor 647, Cy3, Cy5.

[0075] According to the present invention, “image contrast agents” are substances used to increase the contrast of structures or fluids in images. Among the possible contrast agents used in the present invention are iodinated contrast agents, gadolinium derivatives, barium derivatives and radioisotopes.

[0076] It is worth mentioning that, with the present invention, any molecule conjugated to it, this molecule being any fluorophore, any radioisotope or any contrast imaging agent are capable of determining target tumor cells, important in the early diagnosis of tumors.

[0077] As used in the present document, the term “therapeutically effective amount” may mean a sufficient amount of aptamer, anticancer agent, a delivery agent, or composition according to the present invention to inhibit or reduce the number of cancer cells and/or one or more symptoms of cancer. The person skilled in the art will be aware that such an amount will vary depending, for example, on the particular individual and/or the type or severity of the disease level. The term should not be construed to limit the present invention to a specific amount of DNA aptamer.

[0078] The term “individual” is defined herein to include animals, such as mammals. In a preferred embodiment, the individual is a human being.

[0079] Even though the present invention may be susceptible to different embodiments, a preferred embodiment is shown in the drawings and in the following detailed discussion with the understanding that the present description should be considered an exemplification of the principles of the invention and is not intended to limit the present invention to what has been illustrated and described here.

Nucleic Acid Aptamer

[0080] In a first aspect, the invention provides a nucleic acid aptamer which comprises a nucleotides sequence with general formula (1), as follows, or a pharmaceutical acceptable salt thereof: TAGGGAAGAGAAGGACATATGAT-X1-TTGACTAGTACATGACCACTTGA (formula 1), wherein X1 is a nucleotides sequence as defined in any one of the SEQ ID NO: 1-10, SEQ ID NO: 21-30 and SEQ ID NO: 41-50, or a sequence with at least 90% identity to the same which presents a function equivalent to its corresponding. In one embodiment, the nucleic acid is DNA. In another embodiment, the aptamer is for use as a drug or a diagnostic reagent. In another embodiment, the aptamer is for use in the diagnosis and treatment of cancer. In another embodiment, the cancer is selected from the group consisting of prostate, ovarian and breast cancer. In another embodiment, the breast cancer is the triple negative breast cancer.

[0081] In a particular embodiment, the aptamer herein described consists of between 30 and 200 nucleotides, preferably, between 35 and 150 nucleotides, more preferably, between 40 and 100

nucleotides, even more preferably, between 45 and 80 nucleotides.

[0082] The aptamers for use in the diagnosis and treatment of ovarian cancer can be as defined below:

TABLE-US-00001 SEQ ID NO: 1: CATACTCTTTATACTCTCATATTAACCA SEQ
ID NO: 2: TCCCATTCTCTATAATAGTCCCCCACTATA SEQ ID NO: 3:
ACGCTAATATCTGATTAATTACTTTTTTTTG SEQ ID NO: 4:
GGTGTAGTTATTTGATATTCTTTATTGCTG SEQ ID NO: 5:
TGGGATTAGTTTATGTTGTTTGTGTTATT SEQ ID NO: 6:
CACGGATATAATTATACCCTTTTAGTTTGA SEQ ID NO: 7:
CAATACACTCTTTATAAACGTTTCCTTTCA SEQ ID NO: 8:
GTATTGTTTTGTTGTTTCTTGTATAATTCG SEQ ID NO: 9:
TGGTAGTATAGGTTGTTGTTGGTATTTATA SEQ ID NO: 10:
TAGCGTTTTATAAATCTCTCCTTTATTGTG SEQ ID NO: 11:
TAGGGAAGAGAAGGACATATGATCATACTCTCTCATATTA
ACCATTGACTAGTACATGACCACTTGA SEQ ID NO: 12:
TAGGGAAGAGAAGGACATATGATTCCCATTCTCTATAATAGTCCCCAC
TATATTGACTAGTACATGACCACTTGA SEQ ID NO: 13:
TAGGGAAGAGAAGGACATATGATACGCTAATATCTGATTAATTACTTTT
TTTTGTTGACTAGTACATGACCACTTGA SEQ ID NO: 14:
TAGGGAAGAGAAGGACATATGATGGTGTAGTTATTTGATATTCTTTATT
GCTGTTGACTAGTACATGACCACTTGA SEQ ID NO: 15:
TAGGGAAGAGAAGGACATATGATTGGGATTAGTTTATGTTGTTTGTGTTGT
TATTTTGACTAGTACATGACCACTTGA SEQ ID NO: 16:
TAGGGAAGAGAAGGACATATGATCACGGATATAATTATACCCTTTTAGT
TTGATTGACTAGTACATGACCACTTGA SEQ ID NO: 17:
TAGGGAAGAGAAGGACATATGATCAATACACTCTTTATAAACGTTTCCT
TTCATTGACTAGTACATGACCACTTGA SEQ ID NO: 18:
TAGGGAAGAGAAGGACATATGATGTATTGTTTTGTTGTTTCTTGTATAA
TTCGTTGACTAGTACATGACCACTTGA SEQ ID NO: 19:
TAGGGAAGAGAAGGACATATGATTGGTAGTATAGGTTGTTGTTGGTATT
TATATTGACTAGTACATGACCACTTGA SEQ ID NO: 20:
TAGGGAAGAGAAGGACATATGATTAGCGTTTTATAAATCTCTCCTTTAT
TGTGTTGACTAGTACATGACCACTTGA

[0083] It is important to emphasize that the sequences in bold are sequences that are repeated in SEQ ID NO: 11 to 20. The SEQ ID NO: 1 to 10 are the variable regions of SEQ ID NO: 11 to 20.

[0084] The aptamers for use in the diagnosis and treatment of breast cancer can be as defined below:

TABLE-US-00002 SEQ ID NO: 21: ACGAGATCTCGAGGTAAGTCATACTTCGCA
SEQ ID NO: 22: GTCACGAATATGATGAGGCTGACATGTCGG SEQ ID NO: 23:
CGAATTGTCGAACGAGGCTCCTCCGAGGAT SEQ ID NO: 24:
GCTACGAGTTTTGAGGTTTTGCATGGTTCG SEQ ID NO: 25:
GCTACGAGTTTTGAGGTTTTGCATGGTTCG SEQ ID NO: 26:
ACGAGTGTACCGAGGTAAAGTCACTTGGTC SEQ ID NO: 27:
ACGCAGTGCGAATGAGGTAAATCATTTCTC SEQ ID NO: 28:
GGAGTAAGAGCACCCCGGACCGCGCGACCG SEQ ID NO: 29:
CGTAGAACCTGGGATGCATTTACGCGAGTA SEQ ID NO: 30:
GCAACCCGTCCCCCACTAATTTACGCATCA SEQ ID NO: 31:
TAGGGAAGAGAAGGACATATGATACGAGATCTCGAGGTAAGTCATACTT
CGCATTGACTAGTACATGACCACTTGA SEQ ID NO: 32:
TAGGGAAGAGAAGGACATATGATGTCACGAATATGATGAGGCTGACATG

TCGGTTGACTAGTACATGACCACTTGA SEQ ID NO: 33:
TAGGGAAGAGAAGGACATATGATCGAATTGTCTGAACGAGGCTCCTCCGA
GGATTTGACTAGTACATGACCACTTGA SEQ ID NO: 34:
TAGGGAAGAGAAGGACATATGATGCTACGAGTTTTGAGGTTTTGCATGG
TTCGTTGACTAGTACATGACCACTTGA SEQ ID NO: 35:
TAGGGAAGAGAAGGACATATGATGCTACGAGTTTTTGAGGTTTTTGCAT
GGTTCGTTGACTAGTACATGACCACTTGA SEQ ID NO: 36:
TAGGGAAGAGAAGGACATATGATACGAGTGTACCGAGGTAAAGTCACTT
GGTCTTGACTAGTACATGACCACTTGA SEQ ID NO: 37:
TAGGGAAGAGAAGGACATATGATACGCGAGTGCGAATGAGGTAAATCATT
TCTCTTGACTAGTACATGACCACTTGA SEQ ID NO: 38:
TAGGGAAGAGAAGGACATATGATGGAGTAAGAGCACCCCGGACCGCGCG
ACCGTTGACTAGTACATGACCACTTGA SEQ ID NO: 39:
TAGGGAAGAGAAGGACATATGATCGTAGAACCTGGGATGCATTTTCAGCG
AGTATTGACTAGTACATGACCACTTGA SEQ ID NO: 40:
TAGGGAAGAGAAGGACATATGATGCAACCCGTCCCCCCTACTAATTTACG
CATCATTGACTAGTACATGACCACTTGA

[0085] It is important to emphasize that the sequences in bold are sequences that are repeated in SEQ ID NO: 31 to 40. The SEQ ID NO: 21 to 30 are the variable regions of SEQ ID NO: 31 to 40.

[0086] The aptamers for use in the diagnosis and treatment of prostate cancer can be as defined below:

TABLE-US-00003 SEQ ID NO: 41: GCACGTCCCCCACTCCAATAATCGCATCA
 SEQ ID NO: 42: AGACAACGCGTCCCCCACTAACCCCTGTCG SEQ ID NO: 43:
 CGGGTTGTAGTGGTGGCCCCATACCGTATT SEQ ID NO: 44
 CCCCACCCCCCTCTTTGCAGTTGCTCTGG SEQ ID NO: 45:
 CACCGTCCCCCACTGCATCACGATCGTATG SEQ ID NO: 46:
 GGGCTAGGCAAACCCCGTCCCCCACTCTAT SEQ ID NO: 47:
 CGCGGCTCCGAAAGCTCGTTTACCCCGAC SEQ ID NO: 48:
 CAGCCCCAGTCCCCCACTCATTGTTGATCA SEQ ID NO: 49:
 CGGGTGTTTTATGCGGAGGGGTGTTACGGA SEQ ID NO: 50:
 GTCGCGGGAGAGTTTTTGAATTGATGCTG SEQ ID NO: 51:
TAGGGAAGAGAAGGACATATGATGCACGTCCCCCACTCCAATAATCGC
ATCATTGACTAGTACATGACCACTTGA SEQ ID NO: 52:
TAGGGAAGAGAAGGACATATGATAGACAACGCGTCCCCCACTAACCCCT
GTCGTTGACTAGTACATGACCACTTGA SEQ ID NO: 53:
TAGGGAAGAGAAGGACATATGATCGGGTTGTAGTGGTGGCCCCATACCG
TATTTTGACTAGTACATGACCACTTGA SEQ ID NO: 54:
TAGGGAAGAGAAGGACATATGATCCCCACCCCCCTCTTTGCAGTTGC
TCTGGTTGACTAGTACATGACCACTTGA SEQ ID NO: 55:
TAGGGAAGAGAAGGACATATGATCACCGTCCCCCACTGCATCACGATCG
TATGTTGACTAGTACATGACCACTTGA SEQ ID NO: 56:
TAGGGAAGAGAAGGACATATGATGGGCTAGGCAAACCCCGTCCCCCACT
CTATTTGACTAGTACATGACCACTTGA SEQ ID NO: 57:
TAGGGAAGAGAAGGACATATGATCGCGGCTCCGAAAGCTCGTTTACCCC
CGACTTGACTAGTACATGACCACTTGA SEQ ID NO: 58:
TAGGGAAGAGAAGGACATATGATCAGCCCCAGTCCCCCACTCATTGTT
GATCATTGACTAGTACATGACCACTTGA SEQ ID NO: 59:
TAGGGAAGAGAAGGACATATGATCGGGTGTTTTATGCGGAGGGGTGTTA
CGGATTGACTAGTACATGACCACTTGA SEQ ID NO: 60:
TAGGGAAGAGAAGGACATATGATGTCGCGGGAGAGTTTTTGAATTGAT

GCTGTTGACTAGTACATGACCACTTGA

[0087] It is important to emphasize that the sequences in bold are sequences that are repeated in SEQ ID NO: 51 to 60. The SEQ ID NO: 41 to 50 are the variable regions of SEQ ID NO: 51 to 60.

[0088] The production of the aptamer of the invention can be carried out following conventional methods in the art. Non-limiting examples of techniques for producing aptamers include enzymatic techniques such as transcription, recombinant expression systems and, most importantly, standard solid phase (or solution phase) chemical synthesis, all of which are commercially available. When appropriate, for example, in the case wherein the aptamer of the invention comprises nucleic acid variants such as those described above, nucleotide analogs such as analogs that have chemically modified sugars or bases, main chain modifications, etc., the aptamer of the invention will be produced by means of chemical synthesis. The aptamers produced can optionally be purified by methods that are well known in the art.

[0089] For the administration to an individual who needs a nucleic acid aptamer with the ability to specifically recognize tumour lines LNCaP, MDA-MB-321 or OVCAR-3 e which comprises a sequence selected from the group consisting of SEQ ID NO: 11 to SEQ ID NO: 20 or a functionally equivalent variant thereof or a complex which comprises a nucleic acid aptamer with the ability to specifically recognize tumor cell lines LNCaP, MDA-MB-321 or OVCAR-3 and which comprises a sequence selected from the group consisting of SEQ ID NO: 11 to SEQ ID NO: 20 or a functionally equivalent variant thereof and a functional group, said aptamers and complexes can be formulated in suitable compositions.

Composition

[0090] In another aspect, the present invention refers to a composition which comprises at least one aptamer as defined above. In one embodiment, the composition further comprises one or more pharmaceutically acceptable carriers, excipients, or solvents. In another embodiment, the composition additionally comprises one or more additional active ingredients. In another embodiment, the composition is for use in the detection, diagnosis, or treatment of cancer. In another embodiment, the cancer is selected from the group consisting of prostate, ovarian and breast cancer. In one additional embodiment, the breast cancer is the triple negative breast cancer.

[0091] In one embodiment, the composition is formulated with vehicles that will protect said products from rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery system. Biodegradable and biocompatible polymers such as ethylene-vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters and polylactic acid can be used. The methods for preparing such formulations will be obvious to a person skilled in the art.

[0092] The compositions provided by the present invention may be administered to an individual by any suitable route of administration, such as, for example, parenterally.

[0093] The term “parenteral”, in the context of the present invention, includes intravenous, intraperitoneal, intramuscular, or subcutaneous administration. The intravenous form of parenteral administration is generally preferred.

[0094] Furthermore, the compositions provided by the present invention can suitably be administered by pulse infusion, for example, with decreasing doses of the aptamer of the invention. Preferably, dosage is provided by means of injections, more preferably intravenous or subcutaneous injections, depending in part on whether administration is brief or chronic.

[0095] The effective dosage of the anticancer agent can be determined according to weight, age, gender, state of health, diet, frequency of administration, the method of administration, excretion, and severity of a disease. The frequency of administration can be from one to several times a day.

Use of an Aptamer or of a Composition

[0096] In another aspect, the present invention refers to the use of an aptamer as defined above or of a composition as defined above for the manufacture of a drug for the treatment of cancer. In one embodiment, the cancer is selected from the group consisting of prostate, ovarian and breast cancer.

In another embodiment, the breast cancer is the triple negative.

Diagnostic Kit

[0097] In another aspect, the invention refers to a diagnostic kit which comprises the aptamers as defined above or of a composition as defined above.

Method for Detecting or Diagnosing a Tumor

[0098] In another aspect, the invention refers to method for detecting or diagnosing a tumor, which comprises contacting at least one aptamer as defined above or a composition as defined above with a cell, tissue, or sample from an individual, and detecting the binding of the aptamer to the cell, tissue, or sample. In one embodiment, the aptamer is associated with a fluorophore, a contrast agent or a radioisotope. In another embodiment, the cell, tissue, or sample is of prostate, ovarian and breast cancer.

Method for Treating Cancer

[0099] In another aspect, the invention refers to method for treating cancer, which comprises a step of administering to an individual a therapeutically effective amount of an aptamer as defined above or of a composition as defined above.

[0100] As previously mentioned, the present invention is described by the non-limiting examples below, which are merely illustrative. Several modifications and variations of the embodiments are evident to the skilled person in the field, without departing from the spirit and scope of the invention.

EXAMPLES

1. Cell Lines and Cultures

[0101] The prostate tumor and non-tumor cell lines, LNCaP and RWPE-1, respectively, were kindly provided by Dr. Etel Gimba, from INCA. The breast tumor and non-tumor cell lines, MDA-MB-231 and MCF-10A, respectively, and the ovary cell lines, OVCAR-3 and CHO-K1, were acquired commercially by the Rio de Janeiro Cell Bank (Banco de Células do Rio de Janeiro—BCRJ). The description of the lines and their respective genotypes are described in Table 1.

[0102] All the lines were authenticated by the Cell Bank of Rio de Janeiro—Technical Scientific Association Paul Ehrlich APABCAM. In order to facilitate their use in this and other experiments, from the lines received from BCRJ and INCA, the cells were cultured as described below, replicated, frozen and kept in liquid nitrogen (N.sub.2).

TABLE-US-00004 TABLE 1 Panel of tumor and non-tumor cell lines with their genotypes, used in this study for the selection of specific aptamers (www.atcc.org) Non Tumor Genotypes tumor

Genotypes	Tissue lines (STRs)	lines (STRs)
Prostate LNCaP	Amelogenin: X, Y Y CSF1PO: 10, 11 CSF1PO: 13 D13S317: 10, 12 D13S317: 8, 14 D16S539: 11 D16S539: 9, 11 D5S818: 11, 12 D5S818: 12, 15 D7S820: 9.1, D7S820: 10, 11 10.3 THO1: 9 THO1: 8, 9.3 TPOX: 8, 9 TPOX: 8, 11 vWA: 16, 18 vWA: 14, 18	Breast MDA- MB-231 CSF1PO: 12, 13 CSF1PO: 10, 12 D13S317: 13 D13S317: 8, 9 D16S539: 12 D16S539: 11, 12 D5S818: 12 D5S818: 10, 13 D7S820: 8, 9 D7S820: 10, 11 THO1: 7, 9.3 THO1: 8, 9.3 TPOX: 8, 9 TPOX: 9, 11 vWA: 15, 18 vWa: 15, 17
Ovarian OVCAR-3	Amelogenin: X CHO-K1 There is no STR 3 CSF1PO: 11, 12 for CHO-K1 (under D13S317: 12 development) D16S539: 12 D5S818: 11, 12 D7S820: 10 THO1: 9, 9.3 TPOX: 8 vWA: 17	

[0103] The lines were cultivated according to the ATCC (American Type Culture Collection) guidelines. For the LNCaP, MDA-MB-231, OVCAR-3 and CHO-K1 cultures, RPMI medium (Roswell Park Memorial Institute) 1640 (Sigma—6504) was used supplemented with 2 g/L of sodium bicarbonate, 25 mM of Hepes, 10% of bovine fetal serum and 1% of Penicillin and Streptomycin (Sigma—P4333). Cells were passaged weekly with plating of 0.4×10^6 cells in 25 cm.sup.2 culture bottles, maintained at 37° C. in an atmosphere containing 5% CO.sub.2.

[0104] For the cultivation of RWPE-1 cells, Keratinocyte Serum Free Medium (Thermofisher—17005-042) was used, supplemented with 50 mg/L of bovine pituitary extract (BPE), 5.0 µg/L of epidermal growth factor recombinant human (rhEGF) and 1% of Penicillin and Streptomycin

(Sigma—P4333). Cell passages were conducted weekly with plating of 0.4×10^6 cells in 25 cm.^{sup.2} culture bottles, maintained at 37° C. in an atmosphere containing 5% CO._{sub.2}. [0105] For the cultivation of MCF10A, Epithelial Cell Growth Medium (Lonza/Clonectics Corporation—CC-3150—MEGM BulletKit) was used, supplemented with BPE; rhEGF; hydrocortisone; insulin; Penicillin/Streptomycin at 1% (Sigma—P4333) and cholera toxin at 10 ng/mL (Sigma—C8052). The concentrations of BPE, rhEGF, hydrocortisone and insulin were not provided by the manufacturer. Cell passages were conducted weekly with plating of 0.4×10^6 cells in 25 cm.^{sup.2} culture bottles, maintained at 37° C. in an atmosphere containing 5% CO._{sub.2}.

[0106] In all lines in the present study, tests were conducted to verify the presence of mycoplasma in the cultures. The test was standardized by our group through multiplex PCR with the oligonucleotides pool described by Timenetsky et al. (2006) and Peres Cristo (2009). As an endogenous control for the PCR reaction, the set of oligonucleotides for the GAPDH gene was used.

2. Cell SELEX

[0107] In order to select the specific aptamers pool using the Cell SELEX (CS) method, an aptamer library constructed by Trilink Biotechnologies® (USA) was commercially acquired, containing approximately 10^{15} random synthetic oligonucleotides (ssDNA) variants. Aptamers are made up of two flanking sequences of 23 nucleotides (nt), and a central, completely random portion of 30 nt (FIG. 1). The library would have been normalized to actually have all 4 nucleotides present at about 25% frequency in any of the random positions.

[0108] The entire CS standardization process was conducted based on the methodology described in detail by Sefah et al. (2010) (FIG. 2).

[0109] Tumor and control cells were cultured at 5.0×10^6 cells in 60 mm×15 mm Petri dishes with a final volume of 3 mL of specific culture medium for each cell type, as described in FIG. 2, for 24 hours until 90% confluence.

[0110] The ssDNA aptamers library (N30) was eluted in 10 µL of milliQ water to a final concentration of 1 mM. When preparing the aptamers library for final use, 10 µL of the library were diluted in 990 µL of binding buffer (Table 2—binding buffer=wash buffer+1 mg/mL BSA), for a final concentration of 10 µM.

TABLE-US-00005 TABLE 2 Solutions Reagents Concentrations RPMI complete culture medium: RPMI 10.4 g/L NaHCO._{sub.3} 2 g/L Hepes 25 mM Penicillin-Streptomycin 10 U/mL Bovine fetal serum 10% Phosphate buffered saline solution (PBS, pH 7.0) 10x concentrate: NaCl 0.14M KCL 2.7 mM Na._{sub.2}HPO._{sub.4} 6.4 mM KH._{sub.2}PO._{sub.4} 0.88 mM Dulbecco's phosphate buffered saline solution (DPBS, pH 7.0) 10x concentrate: CaCl._{sub.2}•2H._{sub.2}O 1.33 g/L MgCl._{sub.2}•6H._{sub.2}O 1.0 g/L KCl 2.0 g/L KH._{sub.2}PO._{sub.4} 2.0 g/L NaCl 80.0 g/L Na._{sub.2}HPO._{sub.4} (anhydrous) 11.5 g/L Reagents Amounts Wash buffer (Cell SELEX) MgCl._{sub.2} (1M) 1.25 mL Glucose 1.12 g DPBS 250 mL (q.s.) Binding buffer - modified (Cell SELEX) BSA 10 mg DTT 0.5M 10 µL Wash buffer (Cell SELEX) 10 mL (q.s.) Filter and store at 4° C. Reagents Concentrations Polyacrylamide gel 12% SDS-PAGE Tris-HCl (pH 8.8) 1.5M Acrylamide 30% Bis-Acrylamide 0.8% SDS 10% APS 10% TEMED 0.001% Sample buffer (1X) Tris-HCl (pH 6.8) 62.5 mM SDS 2% β-mercapto ethanol 5% Bromophenol blue 0.002% Glycerol 10% Running buffer Laemmli (5X) Tris-base 24.6 mM Glycine 192 mM SDS 3.48 mM Reagents Amounts Transfer buffer (10X) Tris-base 18.90 g Glycine 90 g H._{sub.2}O 1 L (q.s.) Transfer buffer (1X) Transfer buffer 10x 100 mL Absolute ethanol PA 200 mL H._{sub.2}O 1 L (q.s.) Reagents Concentrations TBS buffer (10X) Tris-HCl (pH 7.5) 1M NaCl 1.5M TBS-Tween buffer (1X) Tris-HCl, (pH 7.5) 100 mM NaCl 150 mM Tween 20 0.1%

[0111] First, after dilution, the aptamer library was heated at 95° C. for 5 minutes to break up possible clumps of aptamers and immediately placed on ice to form 3D structures of individual aptamers. Then, the cultures were washed carefully, to avoid the loss of adhered cells, twice with

500 μ L of wash buffer and then they were incubated with 500 μ L of the 10 μ M aptamer library, diluted in 500 μ L of the binding solution.

[0112] For the prostate tumor line, LNCaP, three different incubations were carried out: one at 37° C. (Plate 01—P1) with the aptamer library, another at 4° C. (Plate 02—P2) with the aptamer library, and the third cells plate was incubated only with the binding buffer and without aptamers at 37° C. (Plate 03—P3). It is important to highlight that, for the selection process of specific aptamers for the MDA-MB-231 and OVCAR-3 lines, only incubations at 37° C. were conducted. The cells were incubated with the aptamers for 1 h on a shaker at 50 RPM. After incubation, the supernatant of each plate, where the aptamers not bound to the cells are found, is recovered and stored at -20° C. for future tests, with the aptamers being considered unbound. Then, the cultures were washed 3 times with 500 μ L of wash buffer, carefully to avoid loss of adhered cells. In this step, 500 μ L of milliQ H.sub.2O was added for cell lysis and capture of the aptamers (ssDNAs) that bound to the cells. The material was scraped with a cell scraper, collected, and transferred to a 1.5 ml microtube, heated in a heating block at 95° C. for 10 min and centrifuged at 13000 g for 5 min. The precipitate at the bottom of the tube was discarded and the supernatant, containing the ssDNA aptamers pool resulting from binding to cells, was amplified with different numbers of PCR cycles.

3. Amplification of the First Pool of Selected Aptamers (ssDNAs)

[0113] First round amplification is done under the following conditions (Table 3):

TABLE-US-00006 TABLE 3 Reagents amounts and concentrations for amplifying aptamers using PCR

Reagent	Amount (μ L)	Concentrated Mastermix 2x (Promega®)	(Taq 250 DNA Polymerase - 0.05 U/ μ L)	DNA from the selection of aptamers (P1, P2, 240 P3)	Primer Forward (C.sub.INITIAL = 50 μ M)	5 (C.sub.FINAL = 0.5 μ M)	Primer Reverse (C.sub.INITIAL = 50 μ M)	5 (C.sub.FINAL = 0.5 μ M)
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[0114] A tube with the solutions used in the polymerase chain reaction (PCR) is added in parallel, as a negative control for the reaction (50 l). The total volume is divided equally into 10 (ten) 0.2 mL microtubes, with 50 L for each microtube under the following PCR reaction conditions (Table 4).

TABLE-US-00007 TABLE 4 Cycling conditions for amplification of selected aptamer sequences

Steps	Temperature	Time	Number of cycles
Initial Denaturation	95° C.	5 m	1
Denaturation	95° C.	30 s	10
Forming rings	58° C.	30 s	
Extension	72° C.	30 s	
Final extension	72° C.	3 m	1
Cooling	4-10° C.	∞	1

4. Determination of the Number of PCR Cycles for ssDNA Amplification

[0115] After the PCR for amplification of the ssDNA aptamers selected in the first round of Cell SELEX (CS), a second PCR is performed to choose the most appropriate number of cycles for the amplification of these aptamers. Reagents for PCR and amplification conditions are available in Tables 5 and 6.

TABLE-US-00008 TABLE 5 Reagents amounts and concentrations for amplifying aptamers

Reagent	Amount (μ L)	Concentrated Mastermix 2x (Promega®)	125	DNA from the 1st PCR (P1, P2, P3)	25	Primer Forward (C.sub.INITIAL = 50 μ M)	2.5 (C.sub.FINAL = 0.5 μ M)	Primer Reverse (C.sub.INITIAL = 50 μ M)	2.5 (C.sub.FINAL = 0.5 μ M)	H.sub.2O milliQ	q.s.	(V.sub.FINAL = 250 μ L)	95
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[0116] A tube with the solutions used in the PCR reaction is added in parallel, as a negative control for the reaction (50 L). The total volume is divided equally into 5 (five) 0.2 mL microtubes, with 50 μ L in each. These will be subjected to different numbers of cycles (4, 6, 8, 10 or 12 cycles), under the following PCR reaction conditions:

TABLE-US-00009 TABLE 6 Cycling conditions to determine the best cycle for obtaining ssDNA aptamer sequences

Steps	Temperature	Time	Number of cycles
Initial Denaturation	95° C.	5 m	1
Denaturation	95° C.	30 s	4, 6, 8, 10 or 12 cycles
Forming rings	58° C.	30 s	
Extension	72° C.	30 s	
Final extension	72° C.	3 m	1
Cooling	4-10° C.	∞	1 cycle

[0117] The amplified products were evaluated on a 3% agarose gel using electrophoresis at 80V.

After choosing the cycle with the best amplification quality, an “asymmetric PCR” (or rather, transcription and geometric accumulation reactions) is performed, using only the forward primer to multiply the ssDNA aptamer sequences selected in the first round of CS under the following conditions (Table 7):

TABLE-US-00010 TABLE 7 Reagents amounts and concentrations for obtaining ssDNA aptamer sequences

Reagent	Amount (μl)	Concentration
Concentrated Mastermix 2x (Promega®)	250	DNA from the 1st PCR (P1, P2, P3)
Primer Forward (C.sub.INITIAL)	50	50 μM
Primer Reverse (C.sub.FINAL)	5	0.5 μM
H.sub.2O milliQ q.s.	(V.sub.FINAL = 500 μL)	195

[0118] The total volume is divided equally into 10 0.2 mL microtubes, with 50 L of reaction in each tube. These will be subjected to the number of cycles selected in the previous step (10 cycles), under the following PCR reaction conditions (Table 8):

TABLE-US-00011 TABLE 8 Cycling conditions for obtaining ssDNA aptamer sequences

Steps	Temperature	Time	Number of cycles
Initial Denaturation	95° C.	5 m	1 cycle
Denaturation	95° C.	30 s	x cycles
Forming rings	58° C.	30 s	
Extension	72° C.	30 s	
Final extension	72° C.	3 m	1 cycle
Cooling	4-10° C.	∞	—

[0119] After the multiplication reaction, all the material obtained is gathered in a single microtube and the result is verified on a 3% agarose gel by electrophoretic mobility at 80V. Next, the new library of ssDNA aptamers already partially enriched against the targets is subjected to a desalting process to remove excess salts, primers and other reagents used in the amplification reactions, carried out according to the product manufacturer's protocol, through a purification column for oligonucleotides or small DNA fragments (Illustra™ NAP-5 Sephadex™ G-25 DNA Grade resin—GE Healthcare, UK) and eluted with 500 L of ultrapure H.sub.2O free of DNases and RNases. After the purification process, the ssDNA aptamers were concentrated in a vacuum centrifuge (Speedvac) and then quantified by Qubit (ThermoFisher©) and stored at −20° C. until the next round of Cell SELEX.

[0120] From the third round of CS, we included the step of negative selection, where the ssDNA aptamers pool selected with the tumor cells and eluted are incubated immediately after with the non-tumor cell line (RWPE-1, MCF-10A or CHO-K1). In this step, the ssDNA aptamers selected from the scraping of the tumor cells (supernatant) are incubated on the shaker with the non-tumor cells for 1 h at 37° C. After incubation, the supernatant is collected containing, in principle, aptamers that recognize tumor cells, but those that also recognize normal cells were subtracted. Then, the PCR amplification process begins for a new round of Cell SELEX.

[0121] From the fourth round of CS, in the incubation step of the aptamers with the tumor cells, 10% of bovine fetal serum (Gibco—ThermoFisher©) is added to the binding buffer and throughout the subsequent rounds, the concentration gradually increases until reaching 20% (10, 15 and 20%). This concentration of bovine fetal serum will remain in the binding buffer until the last round of Cell SELEX. Furthermore, from the fourth round onwards, the incubation time with aptamers against tumor cell lines is reduced from 1 hour to 45 minutes and continues to 30 minutes in the next rounds. After 12 rounds of Cell SELEX for the prostate and breast lines and 16 rounds for the ovarian line, the next step consists of aptamer enrichment analysis.

5. Aptamer Pool Enrichment Assessment

[0122] To monitor the process of selection and enrichment of the ssDNA aptamers pool specific to tumor cells, flow cytometry assays were performed.

[0123] Initially, the PCR protocol was conducted for amplification of specific aptamers (forward and reverse), followed by asymmetric PCR, with the forward primer labeled with Fluorescein Isothiocyanate (FITC) to obtain ssDNA aptamer sequences associated with the fluorescent marker. Obtaining labeled aptamers was conducted under the following conditions (Tables 9 and 10):

TABLE-US-00012 TABLE 9 Reagents amounts and concentrations for obtaining ssDNA aptamer sequences associated to FITC marker

Reagent	Amount (μl)	Concentration
Platinum SuperMix High Fi 1x (Thermo)	40	DNA aptamers from the 12th round, amplified
Primer Forward.sub.Fitc	5	

(C.sub.INITIAL = 50 μ M) 0.5 (C.sub.FINAL = 0.5 μ M) H.sub.2O milliQ q.s. (V.sub.FINAL = 50 μ L) 4.5

TABLE-US-00013 TABLE 10 Cycling conditions for obtaining ssDNA aptamer sequences associated to FITC Steps Temperature Time Number of cycles Initial Denaturation 95° C. 5 m 1 cycle Denaturation 95° C. 30 s 30 cycles Forming rings 58° C. 30 s Extension 72° C. 30 s Final extension 72° C. 3 m 1 cycle Cooling 4-10° C. ∞ 1 cycle

[0124] Dose-response linkage testing was performed only with the prostate tumor line, LNCaP. The FITC-labeled aptamers eluted in binding buffer and 20% bovine fetal serum were incubated with 0.4×10^6 cells at concentrations of 5, 10 and 20 M of aptamers for 1 h at 37° C. Then, the cells were centrifuged for 5 minutes at 1200 rpm and the binding buffer was discarded. The cells were washed twice with 500 μ L of wash buffer and 500 L of wash buffer was added to the cells, centrifuged for 5 minutes at 1200 rpm, and the cells were resuspended in 400 μ L of 1 \times PBS buffer to then be analyzed in the cytometer. FACSCalibur (BD) flows from the IOC Cytometry Platform.

[0125] After determining the ideal concentration of aptamers for enrichment assessment, the enrichment profile was evaluated after completing 12 rounds of Cell SELEX for prostate lines (LNCaP and RWPE-1). The conditions for preparing cells with FITC-labeled aptamers are the same as those described in the previous paragraph. Then, the cells were analyzed on the FACSCalibur (BD) flow cytometer of the Flow Cytometry Platform of the Oswaldo Cruz Institute (IOC).

6. Next Generation Sequencing (NGS)

[0126] To improve efficiency in the process of identifying aptamers selected from Cell SELEX and achieve the objective of creating the aptamer bank for the three tumor lines in the present study, next generation sequencing was carried out.

[0127] To conduct this protocol, the ION PGM™ System platform (ThermoFisher Scientific) was used for sequencing of amplified products (Custom Fusion Primers—Ion AmpliSeq™ Technology). The last 5 rounds of each selection were sequenced by Cell SELEX (prostate, breast, and ovary) and for this, PCR amplification of the aptamers pool was performed as described in Tables 11, 12 and 13.

TABLE-US-00014 TABLE 11 Reagents amounts and concentrations for obtaining aptamers sequences Reagent Amount (μ L) Platinum SuperMix High Fi 1x (Thermo) 40 DNA from a set of selected aptamers 5 Primer Forward (C.sub.INITIAL = 50 μ M) 0.5 (C.sub.FINAL = 0.5 μ M) Primer Reverse (C.sub.INITIAL = 50 μ M) 0.5 (C.sub.FINAL = 0.5 μ M) H.sub.2O milliQ q.s. (V.sub.FINAL = 50 μ L) 4.0

TABLE-US-00015 TABLE 12 Reagents amounts and concentrations for obtaining ssDNA aptamers sequences Reagent Amount (μ L) Platinum SuperMix High Fi 1x (Thermo) 40 DNA from the 1st PCR (F/R) 5 Primer Forward (C.sub.INITIAL = 50 μ M) 0.5 (C.sub.FINAL = 0.5 μ M) H.sub.2O milliQ q.s. (V.sub.FINAL = 50 μ L) 4.5

TABLE-US-00016 TABLE 13 Cycling conditions for obtaining ssDNA aptamer sequences Steps Temperature Time Number of cycles Initial Denaturation 95° C. 5 m 1 cycle Denaturation 95° C. 30 s 10 cycles (PCR F/R) 30 cycles (PCR F) Forming rings 58° C. 30 s Extension 72° C. 30 s Final extension 72° C. 3 m 1 cycle Cooling 4-10° C. ∞ 1 cycle

[0128] The samples were quantified (~ 13 ng/ μ L) and sent to Fiocruz's New Generation sequencing platform for the preparation and quantification of libraries generated after the probe fusion reaction for sequencing (FIG. 3). The samples were analyzed in the ION PGM™ System located in the High Complexity Laboratory of the Fernandes Figueira Institute (IFF/FIOCRUZ) of the Fiocruz Technological Platforms Network (VPPCB/FIOCRUZ).

[0129] The analysis of the sequences and the assembly of scripts for data analysis was done in collaboration with Dr. Marcos Catanho from the Bioinformatics Platform at Fiocruz-RJ.

7. Antitumor Activity Test of Selected Aptamers for LNCaP

[0130] In parallel with the aptamer binding assays to tumor cells by flow cytometry, a 30 to 360 minute kinetic was performed to monitor the binding profile of the aptamers pool selected after 12

enrichment cycles, to prostate tumor cells by High Content Screening microscopy (HCS Molecular Devices, LLC), located on the IOC Bioassay Platform. First, 50×10^{sup.3} LNCaP cells were plated in flat-bottomed 96-channel plates. After 24 hours in culture, the cells were incubated with the set of enriched ssDNA aptamers-FITC, at a concentration of 20 M in binding solution. Then, to monitor the kinetics, the tumor cells were analyzed in HCS.

8. Cell Viability Assay

[0131] Following the project rationale, the viability of tumor cells was evaluated after incubation with the 12R ssDNA aptamers pool for 24 hours using the PrestobluTM assay (PrestobluTM Cell Viability Reagent). PrestobluTM is a resazurin-based solution that utilizes the reducing capacity of live cells to quantitatively measure cell proliferation. When cells are alive and healthy, they remain in a reducing environment in the cytosol. Under these conditions, the reagent is reduced to red, highly fluorescent resorufin. Changes can be detected through fluorescence and absorbance measurements.

[0132] First, 50×10^{sup.3} tumor (LNCaP) and non-tumor (RWPE) cells were plated on 96-channel plates and maintained at 37° C. in an atmosphere containing 5% CO₂ for 24 hours. Then, the cells were incubated with the 12R ssDNA aptamers pool, in increasing doses (0.25, 2.5, 12.5, 25, and 50 M) for 24 hours. In addition to these concentrations, the original library used for Cell SELEX was used as a negative control for aptamer activity and, as a positive control, 0.01% Triton was used to induce cell death. After 24 hours of incubation, the aptamers are removed and then 100 µl of PrestobluTM 1× concentrate diluted in culture medium is added to each channel and after 1 hour of incubation, the plates were read on the FlexStation 3 (Molecular Devices®) on the IOC/FIOCRUZ Bioassays platform.

9. Cell Proliferation Assay

[0133] After observing cell viability, the next step was to observe the effect of 12R ssDNA aptamers on the proliferation of prostate tumor (LNCaP) and non-tumor (RWPE) cells. As in the viability assay, 50×10^{sup.3} cells were plated and maintained in culture for 24 hours at 37° C. in an atmosphere containing 5% CO₂. Tumor and control cells were incubated with 12R ssDNA aptamers at increasing doses (0.25, 2.5, 12.5, 25, 50 and 100 M) for 24 hours. Then, the cells were incubated with the Cyquant® reagent for 30 minutes and, subsequently, the plate was read on the FlexStation 3 (Molecular Devices®) located on the IOC/FIOCRUZ Bioassay platform.

10. Assessment of the Cell Death Process

[0134] In order to determine the possible mechanism of cell death, an investigation of cell death by apoptosis was conducted using the TUNEL® (Terminal deoxynucleotidyl transferase Uracil Nick End Labeling) technique. Apoptotic cells can be microscopically recognized through the apparent condensation of chromatin and fragmentation of DNA present in the initial stages of the apoptosis process. DNA cleavage can result in double-stranded and single-stranded DNA (nicks).

[0135] The procedures were conducted according to the description of the commercial kit (In Situ Cell Death Detection Kit, Fluorescein Merk®). 50×10^{sup.3} tumor cells were plated in flat-bottomed 96-channel plates for 24 hours at 37° C. in an atmosphere containing 5% CO₂. Then, the cells were incubated with the 12R ssDNA aptamers pool for 24 h at concentrations of 25 and 50 M. A channel with tumor cells was maintained in culture medium as a negative control.

[0136] After the incubation step with the ssDNA aptamers, the cells were washed once with PBS, and fixed in 4% paraformaldehyde (PFA) for 5 minutes at room temperature. Then, the cells were washed again with PBS and permeabilized with 0.1% Triton x100 in 0.1% sodium citrate buffer for 2 minutes at 4° C. (ice). Then, the cells were washed twice with PBS and the TUNEL reagent was added to the cells for 1 hour at 37° C. After incubation, the cells were washed 3 times with PBS, and then incubated with the DAPI solution (1: 10,000) for 5 minutes at room temperature, washed 1 time with PBS and finally, resuspended in 100 L of PBS for observation under a high content analysis microscope (HCS Molecular Devices) with excitation 450-500 nm and emission at 515-565 nm.

11. Assessment of Death Mechanisms

11.1 Western Blot

[0137] In order to investigate the possible mechanisms of activation of cell death by apoptosis, some proteins of the extrinsic (Caspase 3, 8 and 10) or intrinsic (Caspase 9, Bax and Bcl-2) activation pathway of death by apoptosis were evaluated. Tumor (LNCaP) and non-tumor (RWPE-1) cells were trypsinized and plated in 60 mm×15 mm Petri dishes with a final volume of 3 mL of culture medium. After 24 hours, the cells were incubated with the 12R ssDNA aptamers pool, at 25 μ M for 3 hours at 37° C. in an atmosphere containing 5% CO₂, in order to verify the initial process of death induction. After incubation, the supernatant was collected, and the cells were washed with a wash buffer. Total protein extraction was done by incubating RIPA protein extraction buffer for 30 minutes at 4° C. The material was scraped, collected, and stored at -20° C.

Quantification of total proteins was done by Qubit 2.0® (ThermoFisher Scientific).

[0138] Total proteins (20 μ g) were mixed with the sample buffer, in a final volume of 15 μ L, and heated at 100° C. for 5 minutes. The samples were subjected to 12% polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were separated electrophoretically in Laemmli running buffer at 200V for 1 hour.

[0139] After electrophoresis, the proteins were electrotransferred to a nitrocellulose membrane (Hybond C, GE®), in transfer buffer at 100V for 1 h at 4° C. After transfer, the membrane was stained with a reversible staining kit, MemCode™ Reversible Protein Stain (Pierce), to visualize the efficiency of protein transfer. Subsequently, protein saturation of the membrane was conducted using a solution of 5% skimmed milk in 0.1% TBS-Tween for 2 h at room temperature. After the saturation period, the membrane was washed twice with TBS-Tween for 5 minutes each. Then, the membrane was incubated with primary antibodies (Table 14) diluted in 5% skimmed milk in 0.1% TBS Tween, overnight at 4° C. After incubation, the membrane was washed twice with 0.1% TBS-Tween and twice with 1× concentrated TBS, each washing lasting 5 minutes. Then the membrane was incubated with appropriate secondary antibodies, conjugated anti-rabbit or anti-mouse (animal where the primary antibody was prepared) (Table 13) conjugated to peroxidase, diluted in 5% skimmed milk in 0.1% TBS-Tween, for 1 h at room temperature. After incubation, the membrane was washed again twice with 0.1% TBS-Tween and twice with TBS for 5 minutes.

[0140] The immunostaining was developed by incubating for 5 minutes with SuperSignal® West Peak Chemiluminescent Substrate (Pierce) developing solution according to the manufacturer's instructions and then exposed to the film at different times, developed and fixed in Kodak GBX solutions. The films were scanned using the "GS-800@Calibrated Densitometer" device (BioRad) and images with 600 dpi resolution were generated. Densitometric analysis of the bands was performed using the Amount One® program (version 4.6.9) from BioRad.

TABLE-US-00017 TABLE 14 Dilutions of antibodies used in Western Blot assays

Primary Antibody	Dilution	Manufacturer
CASPASE 1	1:1.000	RD Systems
Anti-Rabbit	Pierce 3 (O/N), 4° C (cat. 269518)	IgG-HRP (cat. 31460)
CASPASE 1	1:500	RD Systems
Anti-Rabbit	Pierce 8 (O/N), 4° C (cat. 84131)	IgG-HRP (cat. 31460)
CASPASE 1	1:1.000	RD Systems
Anti-Mouse	Pierce 10 (O/N), 4° C (cat. 63131)	IgG-HRP (cat. 31430)
CASPASE 1	1:500	RD Systems
Anti-Mouse	Pierce 9 (O/N), 4° C (cat. 8301)	IgG-HRP (cat. 31430)
BAX	1:500	RD Systems
Anti-Rabbit	Pierce (O/N), 4° C (cat. AF820)	IgG-HRP (cat. 31460)
GAPDH	1:20.000	Ambion
Anti-Mouse	Pierce (2 h), Room (cat. AM300)	IgG-HRP (cat. 31430)

[0141] The formulation of the solutions used to prepare the samples and run the gels, as well as the solutions used during the western blot assays are described in Table 2.

11.2 Assessment of Caspase 3 and Annexin V Activation

[0142] To evaluate the Caspase 3 activation, an effector protease in the process of cell death by apoptosis, which acts irreversibly on the instability and breakage of DNA, an assay capable of detecting Caspase 3 activity in live cells in real time was conducted. The NucView® 488 and

CF®594 Annexin V Dual Apoptosis Assay Kit for Live Cells was used, which is an efficient method to observe Caspase 3 activity using the fluorogenic caspase substrate permeable to the cell membrane. Annexin V is a natural phosphatidylserine ligand that, when exposed on the outer membrane, indicates the beginning of the process of activating cell death by apoptosis.

[0143] The commercial kit has an active caspase 3 ligand that emits green fluorescence (488 nm) (represented by the number 03) upon binding. In the case of annexin V, it is labeled with a red fluorescent radical (594 nm) (represented by the number 04), as shown in FIG. 21. In the same assay (FIG. 21), to evaluate the viability of the cells, calcein (435 nm) was used (represented by the number 05), which is capable of measuring both enzymatic activity and cell membrane integrity.

[0144] Initially, 50×10³ tumor and non-tumor cells were plated in 96-well plates for 24 hours. Then, the tumor and control cells were separated into three groups: i) treated with the 12R ssDNA aptamers pool at 25 M; ii) treated with 1 M of Dexamethasone, positive control for induction of death by apoptosis; and iii) untreated (negative control), incubated only with the binding buffer from the kit itself. At the same time, activated caspase 3 (03) markers at 2 M, annexin V (04) (1: 40) and calcein (05) at 20 μM were added to the solution. Next, kinetic monitoring was conducted (20 to 180 minutes) on the Leica confocal microscope, model TCS SP8 (FIGS. 21 and 22).

12. Assessment of Activity of ssDNA Aptamers in 3D Tumor Microspheres

[0145] In order to evaluate the activity of aptamers in a more complex cellular structure and closer to what occurs in vivo, the n3D System technology, developed by Nano3D Biosciences, Inc®, was used to cultivate three-dimensional (3D) spheroids of tumor cells (LNCaP and OVCAR-3) and non-tumor (RWPE-1 and CHO-K1). First, 1×10⁶ cells were coated with the nanospheres for 24 hours in culture in a 6-well plate. According to the manufacturer, the ideal proportion of nanospheres is 1 μL/10,000 cells. After this period, the cells were trypsinized and cultivated at 50×10³ cells in 96-well plates defined as “cell repellent” provided by the manufacturer. The plate was placed on a special base that has a magnetic surface for each well until the formation of the spheroids was complete (2 hours) (FIG. 4). After printing, the base was removed, and the spheroids were cultured for 24 hours. Then, 25 M of 12R ssDNA aptamers were incubated with the 3D tumor and control spheroids for 24 hours.

[0146] In order to evaluate the effect of aptamers on the architecture of spheroids, markers of nucleus (DAPI-1: 10000), actin filaments (Phalloidin-FITC 1: 500) and lysosomes (LysoTracker Red at 5 μM) were used (FIGS. 25, 26 and 27).

Example 1: Construction of the Tumor-Specific Aptamers Library

[0147] The objective is to select specific aptamers, which recognize molecules differentially expressed on the surface of tumor cells, through the Cell SELEX methodology, to identify tumor-specific aptamers for prostate (LNCaP), breast (MDA-MB-321) and ovarian (OVCAR-3) tumor lines, and create a bank of aptamers with potential use in diagnosis and target-specific therapy of the tumors described above. These results are intended to contribute to the diagnosis and development of new therapeutic strategies with minimal side effects on healthy cells.

1.1: Culture of Tumor Cells and Non-Tumor Cells (Control):

[0148] The morphological characteristics of each cell type in culture can be seen in FIG. 5. Prostate tumor cells, LNCaP (FIG. 5A), are poorly adherent epithelial cells derived from lymph node metastases, have an elongated morphology, and an aggregated profile in culture (Horoszewicz et al., 1983). RWPE-1 (FIG. 5B) are non-tumor prostate epithelial cells, derived from the peripheral zone of the normal adult human prostate, which are more spread out in culture, adherent and do not show an aggregated profile in culture (Bello et al., 1997). OVCAR-3 ovarian tumor cells (FIG. 5C) are adherent epithelial cells, derived from malignant ascites from a patient with progressive ovarian adenocarcinoma and form colonies in cultures with low cell density (Hamilton et al., 1983). Cho-K1 cells (FIG. 5D), were used as non-tumor ovarian control cells, are adherent epithelial cells, fast growing in culture and derived from hamster ovary biopsy (Puck et al., 1958). The breast tumor line, MDA-MB-231 (FIG. 5E), of adenocarcinoma origin has epithelial morphology, derived from

a lung metastatic site, and is elongated and adherent cells in culture (Brinkley et al., 1980). The non-tumor breast cell line, MCF10-A (FIG. 5F), are slow-growing adherent cells derived from the mammary gland (Soule and McGrath, 1991).

1.2 Standardization of the Aptamer Selection Method

[0149] The Cell SELEX methodology used was based on the methods described by Sefah et al., 2010. The procedure is based on successive incubations with the cells of interest, starting with the aptamer library in the first round; and from the second round onwards, the amplified product from the previous round is used, eliminating the sequences recognized by the non-tumor control cells of each tumor type, as described in the methodology.

[0150] A screening and selection step of aptamers that specifically recognize each tumor type was conducted with prostate (LNCaP), breast (MDAMB-231) and ovarian (OVCAR-3) lines by Cell SELEX. Enrichment of specific aptamers was completed after 12 rounds for LNCaP and MDA-MB-231 and 16 rounds for OVCAR-3.

1.3 Cell SELEX: Selection of Aptamers for Prostate Tumor Line

[0151] 12 rounds of Cell SELEX were performed with LNCaP cells, with an incubation temperature (37° C.) (FIG. 6).

[0152] Negative selection with non-tumor prostate control cells, RWPE-1, was conducted from the 3rd round onwards. The result that represents the amplification to evaluate the best number of PCR cycles of the aptamers pool selected in the 12th round of Cell SELEX is shown in FIG. 6A and in this case, it was determined that 10 PCR cycles is the best amplification condition for the aptamers pool for LNCaP. The next step consists of the selective amplification assay (ssDNA), characterized by being an asymmetric PCR where only one of the strands is amplified (FIG. 6B).

[0153] Monitoring the selection and enrichment of aptamers specific to tumor cells was conducted using flow cytometry assays (FIG. 7). A dose-response linkage test (5, 10 and 20 M) was performed only with the prostate tumor line, LNCaP (FIG. 7). Under these conditions, it was found that the best concentration for binding tumor cells recognized by specific aptamers was 20 μ M, which was then the concentration chosen for subsequent enrichment assays for all cell types.

[0154] In order to decide to interrupt the aptamer selection procedure, the aptamers selected in the 6th, 8th, 10th and 12th rounds of selection (6R-12R) were incubated with tumor cells and prostate control. In this assay, it was possible to observe the enrichment of binding of the aptamer pool to tumor cells and decreased binding to non-tumor cells in the last three rounds of selection (FIG. 8). It was observed that the binding of the aptamers selected in 12R was specific to tumor cells with no binding to non-tumor cells, indicating that enrichment and selection had reached the limit and thus, Cell SELEX was interrupted.

[0155] After completing 12 rounds of Cell SELEX, using FITC-labeled aptamers, the enrichment profile of aptamers for prostate cell lines (LNCaP and RWPE-1) was evaluated. In this context, it was confirmed that the 12 rounds of Cell SELEX were sufficient for the enrichment of aptamers (FIG. 8).

1.4 Cell SELEX: Selection of Aptamers for Breast Tumor Line

[0156] As described for prostate tumors, 12 rounds of incubation of aptamers were conducted in MDA-MB-231 cells at 37° C., with negative selection conducted from the 3rd round with non-tumor control breast cells, MCF-10A. The procedures for incubating the aptamer library in tumor cells are similar regardless of the tumor type.

[0157] In the PCR experiment to verify better cycling, 12 cycles were chosen for the preparative PCR assay (selective amplification) as they presented an amplified product and a single band (FIG. 8). In the selective amplification assay using primer F alone, preparative PCR, specific ssDNA molecules were obtained (FIG. 8) which were used in the subsequent round.

[0158] In the same way as performed for prostate cells, to decide to interrupt the aptamer selection procedure, the aptamers selected in the 6th, 8th, 10th and 12th round of selection (6R-12R) were incubated with the breast tumor and control cells. We observed an enrichment of the binding of the

aptamer pool to tumor cells and a decrease in binding to non-tumor cells in the last two rounds of selection. We observed the binding of 12R aptamers specifically to tumor cells with no binding to non-tumor cells, indicating that enrichment and selection had reached the limit and, therefore, Cell SELEX was interrupted (FIG. 10).

1.5 Cell SELEX: Selection of Aptamers for Ovarian Tumor Line

[0159] 16 rounds of incubation of aptamers were conducted in OVCAR-3 cells at 37° C., with negative selection conducted from the 3rd round with non-tumor ovarian control cells, CHO-K1. The procedures for incubating the aptamer library in tumor cells are similar regardless of the tumor type. The representative result of the 16 rounds is shown in FIG. 11. Based on the cycle PCR results, 12 cycles were chosen as the best for conducting the preparative PCR (FIG. 11) to obtain ssDNA molecules, which were then used in the subsequent round.

[0160] In the same way as conducted for prostate and breast cells, to decide to interrupt the aptamer selection procedure. First, we evaluated enrichment in the 12th round, as was evaluated for the prostate and breast lines. In this context, the remaining connection to non-tumor cells was observed with the aptamers obtained in the 12th round of selection, which led us to continue the selections until the 16th round of Cell SELEX. The enrichment assessment of the selected aptamers was conducted in the 10th, 12th, 14th and 16th round of selection (10R 16R) with ovarian tumor and control cells. With aptamers obtained in the 16th round, we observed an enrichment of the binding of the aptamer pool to tumor cells and a decrease in binding to non-tumor cells in the last two rounds of selection. We observed the binding of 16R aptamers specifically to tumor cells with low binding to non-tumor cells, indicating that enrichment and selection had reached the limit and, therefore, Cell SELEX was interrupted (FIG. 12).

1.7 High-Throughput Sequencing of Selected Aptamers

[0161] We chose to use the new generation sequencing method Ion Torrent™ Personal Genome Machine™ (PGM) (ThermoFisher Scientific™) as a tool to achieve the objective of creating a bank of specific aptamers against prostate, breast and ovarian lines.

LNCaP

[0162] In order to identify the aptamers sequences selected for LNCaP cells, sequencing of the last five rounds of aptamer selection was conducted at 37° C. and 4° C. using the Ion Torrent Personal Genome Machine (PGM) next generation sequencing technology (ThermoFisher Scientific). Table 15 describes the summary of the sequence analyzes obtained in the last five rounds (R8-R12) of selection at the two different temperatures. To facilitate understanding, the aptamers selected from incubation at 37° C. were named LNCaP1, while those selected from incubation at 4° C. were named LNCaP2. Regarding aptamers from LNCaP1, it was observed that the number of total sequences obtained varied throughout the rounds, reaching 7045 sequences in the last round (Table 15). Likewise, the number of total aptamer sequences originating from LNCaP2 also varied throughout the rounds, reaching 10057 sequences in the last round (Table 16). It was also observed that the percentage of sequences obtained that presented the 2 primer recognition adapters and that were in the expected size of 30 nucleotides also varied throughout the rounds (Tables 15 and 16): of the 7045 sequences obtained in the last round of selection of LNCaP1, 4658 were within the expected standard, with two adapters and a random sequence size of 30 nucleotides, representing 66% of the total sequences obtained. Of the 10057 sequences obtained in the last round of LNCaP2 selection, 7125 were within the expected standard, with two adapters and a random sequence size of 30 nucleotides, representing 71% of the total sequences obtained.

TABLE-US-00018 TABLE 15 Analysis of aptamer sequences for LNCaP1 obtained through NGS sequencing using the Ion Torrent method

Selection rounds	R8	R9	R10	R11	R12	Total sequences obtained
Sequences with 2 adaptors	7358	10648	13895	7680	7045	4847 6505 8805 4100 4695
Size ~30 b	4818	6459	8735	4064	4658	











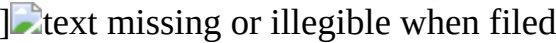
TABLE-US-00019 TABLE 16 Analysis of aptamer sequences for LNCaP2 obtained through NGS sequencing using the Ion Torrent method

Selection rounds	R8	R9	R10	R12	Total sequences
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obtained 10445 9885 13802 10057 Sequences with 2 adaptors 7542 6872 9762 7210 Size ~30 b 7482 6796 9670 7125

[0163] The 10 aptamer sequences with the highest enrichment in the last round of selection at 37° C. (Table 17) were selected to conduct the structural profile considering the structure with the lowest free energy, using the Mfold program.

TABLE-US-00020 TABLE 17 Mfold structural prediction analysis of LNCaP1 aptamers

Number of sequences	Aptamer	observed in 2D	Sequence Code	Free energy (Ag)	round	12 structure			
LNCaP1.1	-4.83 kcal/mol	3	[00001]		LNCaP1.2	-5.53 kcal/mol			
2	[00002]		LNCaP1.3	-2.78 kcal/mol	2	[00003]			
	LNCaP1.4	-3.35 kcal/mol	2	[00004]		LNCaP1.5	-4.79 kcal/mol		
2	[00005]		LNCaP1.6	-6.55 kcal/mol	2	[00006]		LNCaP1.7	-2.59 kcal/mol
2	[00007]		LNCaP1.8	-3.97 kcal/mol	2	[00008]		LNCaP1.9	-1.27 kcal/mol
2	[00009]		LNCaP1.10	-4.46 kcal/mol	2	[00010]		[00011]	












[0164] The identification of the aptamer sequences selected in the last five selection rounds conducted for MDA-MB-231 cells was conducted using the Ion Torrent new generation sequencing technique. Table 19 describes the summary of the analysis of the sequences obtained in the last five rounds (R8-R12). It was observed that the number of total sequences obtained varied throughout the rounds, reaching 10,444 sequences in the last round (Table 18). Furthermore, we observed that the percentage of sequences obtained that presented the 2 primer recognition adapters and that were in the expected size of around 30 nucleotides also varied throughout the rounds: of the 10444 sequences obtained in the last round of selection, 7085 presented within the expected standard, with two adapters and a random sequence size of around 30 nucleotides, representing 68% of the total sequences obtained.

TABLE-US-00021 TABLE 18 Analysis of aptamer sequences for MDA-MB-231 obtained through NGS sequencing using the Ion Torrent PGM™ method

Selection rounds	R8	R9	R10	R11	R12
Total sequences obtained	7135	6157	6489	6442	10444
Sequences with 2 adaptors	4909	3768	3519	2651	7146
Size ~30 b	4889	3752	3487	2629	7085

[0165] The 10 aptamer sequences with the highest enrichment in the last round of selection were selected to conduct the structural profile considering the structure with the lowest free energy, using the Mfold program (Table 19).

TABLE-US-00022 TABLE 19 Mfold structural prediction analysis of MDA-MB-231 aptamers

Number of sequences	Aptamer	observed in 2D	Sequence Code	Free energy (Ag)	round	12 structure	
MDA-MB231.1	-5.36 kcal/mol	100	[00012]		MDA-MB231.2	-6.15 kcal/mol	
29	[00013]		MDA-MB231.3	-3.69 kcal/mol	8	[00014]	
MDA-MB231.4	-1.84 kcal/mol	7	[00015]		MDA-MB231.5	-1.84 kcal/mol	
5	[00016]		MDA-MB231.6	-4.71 kcal/mol	3	[00017]	
MDA-MB231.7	-4.78 kcal/mol	3	[00018]		MDA-MB231.8	-1.91 kcal/mol	
2	[00019]		MDA-MB231.9	-2.17 kcal/mol	2	[00020]	
MDA-MB231.10	-4.73 kcal/mol	2	[00021]		[00022]		

OVCAR-3

[0166] The identification of the aptamer sequences selected in the last five rounds was conducted for OVCAR-3 cells using the Ion Torrent PGM new generation sequencing technique. Table 21 describes the summary of the sequence analysis obtained in the last five rounds (R12-R16). It was observed that the number of total sequences obtained varied throughout the rounds, reaching 17110 sequences in the last round (Table 20). Furthermore, it was observed that the percentage of sequences obtained that presented the 2 primer recognition adapters and that were in the expected size of around 30 nucleotides also varied throughout the rounds of the 17110 sequences obtained in the last round of selection, 11067 presented within the expected standard, with the two adapters and












size of the random sequence around 30 nucleotides, representing 65% of the total sequences obtained.

TABLE-US-00023 TABLE 20 Analysis of aptamer sequences for OVCAR-3 obtained through NGS sequencing using the Ion Torrent method

Selection rounds	R12	R13	R14	R15	R16	Total
Sequences	12565	17229	12505	12861	17110	obtained
Sequences with	8663	12673	9222	9033	11313	2
adaptors	Size ~30 b	8537	12472	9088	8699	11067

[0167] The 10 aptamer sequences with the highest enrichment in the last round of selection were selected to conduct the structural profile considering the structure with the lowest free energy, using the Mfold program (Table 21).

TABLE-US-00024 TABLE 21 Mfold structural prediction analysis of OVCAR-3 aptamers

Number of sequences	Aptamer observed in 2D	Sequence Code	Free energy (Ag)	round	16 structure
OVCAR-3.1	-2.03 kcal/mol	16 [00023]		OVCAR-3.2	-5.31 kcal/mol
12 [00024]		OVCAR-3.3	-0.49 kcal/mol	6 [00025]	
OVCAR-3.4	-0.37 kcal/mol	6 [00026]		OVCAR-3.5	-0.91 kcal/mol
6 [00027]		OVCAR-3.6	-0.99 kcal/mol	6 [00028]	
OVCAR-3.7	-1.62 kcal/mol	5 [00029]		OVCAR-3.8	-1.72 kcal/mol
5 [00030]		OVCAR-3.9	-0.10 kcal/mol	5 [00031]	
OVCAR-3.10	-1.26 kcal/mol	5 [00032]		[00033]	

Example 2: Antitumor Activity Test of Selected Aptamers for LNCaP

[0168] Firstly, it is important to highlight that the results obtained from this step come from aptamers selected by Cell SELEX, with incubation at 37° C., against the prostate tumor line (LNCaP).

2.1: Cell Morphology

[0169] In parallel with the aptamer binding to tumor cells assays by flow cytometry, we also carried out a 30 to 360 minute kinetics to follow the binding profile of the aptamer pool to prostate tumor cells by High Content Screening microscopy (HCS Molecular Devices, LLC), located on the IOC Bioassay Platform. We observed cells labeled with the FITC aptamers at increasing concentrations from 1 to 100 M from the first 30 minutes to 240 minutes (4 hours) of incubation with the aptamers. After 360 minutes, no more aptamer binding to the cells was observed (data not shown). An interesting observation was that the cells positively marked with aptamers were rounded with a loss of adhesion profile, possibly indicating a change in cell viability (FIG. 13).

[0170] In a more detailed analysis of the region highlighted in FIG. 14 (arrows), we can see cells that were still spread out and marked with aptamers-FITC at 1 M at 30 and 60 minutes, and after 120 and 240 minutes it is possible to notice the change in cell profile to rounded cells. In the other aptamer concentrations, the labeled cells already had a rounded profile from the initial analysis times.

[0171] After confirming that the selected aptamers bound to prostate tumor cells (LNCaP), and that they seemed to have some effect on the viability of the cells, cell morphology was assessed after incubation with the 12R aptamer pool in a dose-dependent manner (0.25 to 50 µM) for 24 hours. We observed that after 24 hours of incubation with the aptamer pool, the cells were retracted, with a rounded profile from the concentration of 2.5 M, with a potentially lethal effect at the concentration of 50 µM, in which few adhered cells were observed, and all were rounded (FIG. 15).

2.2: Cells Viability

[0172] To evaluate the viability of LNCaP cells after incubation with the 12R aptamer pool, in increasing doses (0.25 to 50 µM) for 24 hours, an assay was performed with PrestoBlue™ (PrestoBlue™ Cell Viability Reagent). PrestoBlue™ is a resazurin-based solution that utilizes the reducing capacity of live cells to quantitatively measure cell viability. When cells are alive and healthy, they remain in a reducing environment in the cytosol. Under these conditions, the reagent is reduced to red, highly fluorescent resorufin. A negative control of the activity of the aptamer pool was conducted, using the original aptamer library prior to the Cell SELEX (library) and no

significant effect was observed (FIGS. **16A** and **16B**). Incubation of prostate tumor cells with the 12R aptamer pool for 24 hours led to a significant loss of cell viability at concentrations of 12.5; 25 and 50 μM , reaching more than 50% loss of viability, with an inhibitory concentration value of 42 μM ($\text{IC}_{50}=42 \mu\text{M}$) and no effect on non-tumor control cells, RWPE-1 (FIG. **16B**).

2.3: Cell Proliferation

[0173] The effect of compounds with anti-tumor activity on cell proliferation is very relevant and therefore we decided to assess whether the aptamers selected in the 12th round of selection have an effect on the proliferation of prostate tumor cells. Prostate tumor and non-tumor cells were incubated with 12R ssDNA aptamers at increasing doses (0.25 to 100 μM) for 24 hours. After 24 hours in contact with the aptamers pool, the cells were incubated for 30 minutes with the Cyquant® reagent to determine the cell proliferation profile. It was observed that aptamers at 25, 50 and 100 μM inhibited the proliferation of prostate tumor cells by around 50%, without significant effect on non-tumor cells. (FIG. **17**).

2.4: Induction of Cell Death

[0174] Based on the observed results of potential anti-proliferative effect and inhibition of tumor cell viability after incubation with the 12R ssDNA aptamer pool, we investigated the induction of cell death by apoptosis using the TUNEL® technique (Terminal deoxynucleotidyl transferase Uracil Nick End Labeling). In qualitative analysis, we observed that tumor cells incubated with the 12R ssDNA aptamers pool at 25 and 50 μM for 24 hours were in the process of death by apoptosis, as seen in the images in FIG. **18**, in which the positively stained green cell nuclei are observed, indicating activation of the apoptosis process.

2.5: Cell Death Induction Mechanisms

Assessment of Activation of the Intrinsic and Extrinsic Cell Death Pathways by Apoptosis

[0175] In order to understand the possible pathways for inducing death by apoptosis, Western Blot assays were performed for some of the upstream effectors for the extrinsic (Caspase 8 and 10) or intrinsic (Caspase 9, Bax and Bcl-2) activation pathways of death by apoptosis. As the upstream process of inducing death must occur before the final effect of observing the death of tumor cells, we conducted the assay before 24 hours, assessing the 3-hour period for the activation of the death-inducing signaling pathway, since we had observed the complete entry of aptamers by tumor cells in internalization assays. Thus, prostate tumor and non-tumor cells were then incubated with the 12R ssDNA aptamers pool for 3 hours in order to verify the initial process of death induction.

[0176] The results demonstrate increased expression of Caspase 10 in prostate tumor cells after incubation with the 12R ssDNA aptamer pool (FIG. **19A**). Furthermore, it was not possible to observe bands in the assays conducted to detect Caspase 8 in both tumor and non-tumor cells (data not shown). Weak detection for Caspase 9 was observed in prostate tumor cells, but there was no change upon incubation with the 12R ssDNA aptamers pool (FIG. **19B**). No band referring to Caspase 9 and 10 was detected in RWPE-1 cells (FIGS. **19A** and **B**), indicating no induction of death in non-tumor cells. Bax detection was observed in tumor and non-tumor cells, without any effect of the 12R ssDNA aptamer pool (FIG. **19C**). It was also not possible to observe bands in the assays performed to detect Bcl-2 in both tumor and non-tumor cells (data not shown). Therefore, it is concluded that the induction of the apoptosis process observed by the TUNEL assay after addition of the 12R ssDNA aptamer pool must be caused by the extrinsic activation pathway, via Caspase 10.

Assessment of Factors Secreted by the LNCAP Cell as Potential Death Inducers

[0177] To characterize the possible initial event activating the extrinsic pathway, TRAIL was measured using the ELISA assay, secreted into the supernatant of tumor and non-tumor cells with or without the addition of the 12R ssDNA aptamer pool for 3 hours. An increase in TRAIL secretion by prostate tumor cells was observed in response to the addition of the 12R aptamer pool (FIG. **20**) and no effect on non-tumor cells (RWPE-1).

Assessment of Annexin V Expression and Caspase 3 Activation

[0178] Since the 12R ssDNA aptamer pool induced a significant loss of cell viability, followed by death in tumor cells by apoptosis, we developed an assay capable of detecting caspase 3 activity in live cells in real time. The NucView® 488 and CF®594 Annexin V Dual Apoptosis Assay Kit for Live Cells is an efficient method for observing caspase 3 activity using the fluorogenic caspase substrate permeable to the cell membrane. Annexin V is a natural phosphatidylserine ligand which, when exposed on the membrane, indicates the start of the cell death activation process by apoptosis. Caspase 3 activation and Annexin V binding were followed in kinetics starting at 20 minutes up to 180 minutes of incubation with the 12R ssDNA aptamers pool in prostate tumor cells, LNCaP (FIG. 21A-I). In addition to using the 12R ssDNA aptamer pool, we used 1 M dexamethasone as a positive control for death induction (FIGS. 22A and B). In the first 20 minutes it was possible to observe a large number of viable tumor cells that were marked with calcein blue (05), (FIG. 21) which is capable of measuring both enzymatic activity and cell membrane integrity (FIG. 21A).

[0179] In addition, at 20 minutes of incubation of the aptamer pool, we observed that some cells were already marked by Annexin V (04), indicating the translocation of phosphatidylserine into the extracellular medium (FIG. 21, in detail in J, (04)), indicative of the start of the cell death induction process. After 40 minutes of incubation, in addition to the marking of Annexin V (04) on the surface of the cells, we observed the activation of caspase 3 (03) in the cells, FIG. 21, in detail in K, arrow (03). The LNCaP cells activated caspase 3 (03) rapidly, showing a 4× increase in relation to the initial time with just 40 minutes of incubation with the 12R ssDNA aptamers at 25 M (FIG. 22A). The peak activity of the 12R ssDNA aptamer pool was at 80 minutes of incubation, reaching activation 8 times greater than the INITIAL evaluation time (FIG. 22A). On the other hand, the peak of activity in LNCaP cells induced by 1 M dexamethasone was observed at 160 minutes, reaching 2 times more activity than the initial time (FIG. 22A). Interestingly, RWPE-1 cells were more susceptible to activity by dexamethasone (light gray), with an increase in caspase 3 activation over the period studied, showing around 8 times greater activation at 160 minutes of the assay (FIG. 22B).

[0180] These data confirm the induction of death by apoptosis of prostate tumor cells, LNCaP, by the action of the 12R ssDNA aptamer pool, with the participation of caspase 3 potentially activated by the extrinsic death induction pathway, via caspase 10 and secretion of TRAIL.

Example 3: Evaluation of the Activity of ssDNA Aptamers in 3D Tumor Microspheres

[0181] To assess whether the ssDNA aptamers would maintain their activity in a more complex cell structure, we made 3D spheroids in both tumor and non-tumor prostate and ovarian cell lines. The microspheres were grown using n3D System technology, developed by Nano3D Biosciences, Inc.

[0182] In the first instance, the potential of the cells included in the study to form spheroid structures with Nano3D technology was assessed. To this end, tumor, and non-tumor ovarian cells in different amounts (25×10^3 , 50×10^3 and 75×10^3) incubated with the magnetized nanoparticles were submitted to a magnetic plate (Bioprinting) for two hours. Tumor and non-tumor prostate cells were induced to form spheres only with 50×10^3 cells. Spheroid formation was observed in all the cell lines herein analyzed (OVCAR-3 and CHO-K1, FIG. 23 and LNCaP and RWPE, FIG. 24).

[0183] After 24 hours in culture, the 3D spheroids of ovarian and prostate tumor cells were incubated for another 24 hours with the ssDNA aptamer pool selected specifically for each tumor type. In the comparative analyses of the spheroids of OVCAR-3 and CHO-K1 cells, it was observed that OVCAR-3 showed a 40% larger size when compared to the spheroids of non-tumor control ovarian cells (CHO-K1). With regard to the analysis of LNCaP and RWPE-1, it was observed that the spheroids of LNCaP cells were 2.5 times larger when compared to the spheroids of non-tumor control prostate cells (RWPE-1). In both cases, they were cultured with the same initial cell number (FIG. 25 and FIG. 26). Furthermore, a subtle decrease in the ovarian tumor microsphere was observed after 24 hours of incubation with the ssDNA aptamers selected at 50 and

25 μM (FIG. 25), which can also be observed with prostate tumor spheroids (FIG. 26). [0184] In addition, to assess the effect of aptamers on the architecture of spheroids, markers were used for the nucleus (DAPI), actin filaments (Phalloidin-FITC) and lysosomes (LysoTracker-Red); the latter to assess whether the addition of aptamers would increase lysosomal activity, since aptamers can be internalized via lysosomes. In the ovarian tumor spheroids, an increase in actin filament staining was observed, showing a change in the cytoskeleton structure and an increase in lysosomal activity observed by the higher intensity of staining by LysoTracker after incubation with the aptamer pool (FIG. 27). In prostate tumor spheroids, the aptamer pool also appears to increase labeling for actin filaments and also causes an increase in lysosomal activity observed by LysoTracker labeling. In addition, rupture of the microsphere was observed, where it was possible to see evident extravasation of cells (FIG. 27).

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Claims

1. A nucleic acid aptamer comprising a nucleotide sequence with general formula (1) as follows below or a pharmaceutical acceptable salt thereof: TAGGGAAGAGAAGGACATATGAT-X1-TTGACTAGTACATGACCACTTGA (Formula 1), wherein: X1 is a nucleotide sequence as defined in any one of the SEQ ID NO: 1-10, SEQ ID NO: 21-30 and SEQ ID NO: 41-50 or a sequence with at least 90% identity to the same which presents a function equivalent to its corresponding.
2. The aptamer of claim 1 wherein the nucleic acid is DNA.
3. The aptamer of claim 1 wherein it is for use as a drug or a diagnostic reagent, preferably, in the diagnosis and treatment of cancer.
4. (canceled)
5. The aptamer of claim 3 wherein the cancer is selected from the group consisting of prostate, ovarian and breast cancer, optionally, wherein the breast cancer is triple negative breast cancer.
6. (canceled)
7. A composition comprising at least one aptamer of claim 1 and one or more pharmaceutically acceptable carriers, excipients or solvents, optionally comprising one or more additional active ingredients.
8. (canceled)
9. (canceled)
10. The composition of claim 7 wherein it is for use in the detection, diagnosis, or treatment of cancer.
11. The composition of claim 7 wherein the cancer is selected from the group consisting of prostate, ovarian and breast cancer, optionally, the breast cancer is triple negative breast cancer.
- 12.-15. (canceled)
16. A diagnostic kit comprising the aptamer of claim 1.
17. A method for detecting or diagnosing a tumor comprising: contacting at least one aptamer of claim 1 or a composition with a cell, tissue, or sample from an individual, and detecting the binding of the aptamer to the cell, tissue, or sample.
18. The method of claim 17 wherein the aptamer is associated with a fluorophore, to an image

contrast agent or to a radioisotope.

19. The method of claim 17 wherein the cell, tissue, or sample is of prostate, ovarian or breast cancer.

20. A method for treating cancer, comprising a step of administering to an individual a therapeutically effective amount of an aptamer of claim 1.

21. A diagnostic kit comprising the composition of claim 7.

22. A method for detecting or diagnosing a tumor comprising: contacting at least a composition of claim 7 with a cell, tissue, or sample from an individual, and detecting the binding of the aptamer to the cell, tissue, or sample.

23. The method of claim 22 wherein the aptamer is associated with a fluorophore, to an image contrast agent or to a radioisotope.

24. The method of claim 23 wherein the cell, tissue, or sample is of prostate, ovarian or breast cancer.

25. The method of claim 20 wherein the cancer is selected from the group consisting of prostate, ovarian and breast cancer, optionally, wherein the breast cancer is triple negative breast cancer.

26. A method for treating cancer comprising a step of administering to an individual a therapeutically effective amount of a composition of claim 7.

27. The method of claim 26 wherein the cancer is selected from the group consisting of prostate, ovarian and breast cancer, optionally, wherein the breast cancer is triple negative breast cancer.
