Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Nuclease activity as specific biomarker for breast cancer

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Table S1. Oligonucleotide probe sequences.

Seq Name	Sequence
DNA	FAM// TCTCGTACGTTC //TQ2
RNA	FAM// **ucucguacguuc** //TQ2
All 2'-OMe	FAM// mUmCmUmCmGmUmAmCmGmUmUmC //TQ2
Pyr 2'-OMe	FAM// mUmCmUmC**g**mU**a**mC**g**mUmUmC //TQ2
Pur 2'-OMe DNA	FAM// TmAmAC mG TmAC mGmG TC //TQ2
Pur 2'-OMe RNA	FAM// **u**mAmA**c**mG**u**mA**c**mGmG**uc** //TQ2
All 2'-F	FAM// fUfCfUfCfGfUfAfCfGfUfUfC //TQ2
Pyr 2'-F	FAM// fUfCfUfC**g**fU**a**fC**g**fUfUfC //TQ2
Pur 2'-F DNA	FAM// TfAfAC fG TfAC fGfG TC //TQ2
Pur 2'-F RNA	FAM// **u**fAfA**c**fG**u**fA**c**fGfG**uc** //TQ2

Uppercase red TACG = DNA
Lowercase blue uacg = RNA
m = 2'-O-Methyl modifcation
f = 2'-Fluoro modification.

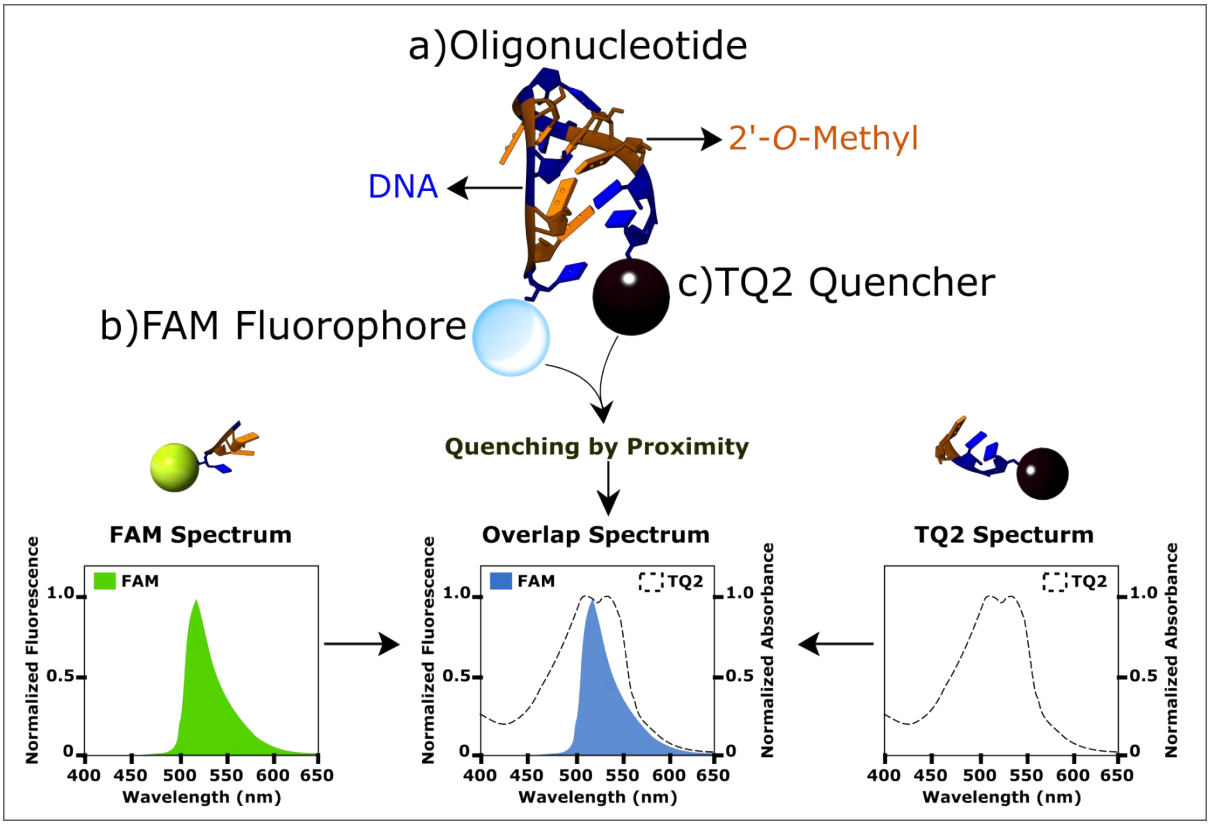

Figure S1. Description and spectrum information of the cancer probe. Synthesized probe with the three main components: a) 2'-O-Methyl chemically modified oligonucleotide, b) FAM fluorophore at the 5´-end , and c) TQ2 quencher at the 3´-end. In this scheme, the probe is quenched by proximity of the FAM and TQ2 as shown in the the overlap spectrum (middle, lower panel).

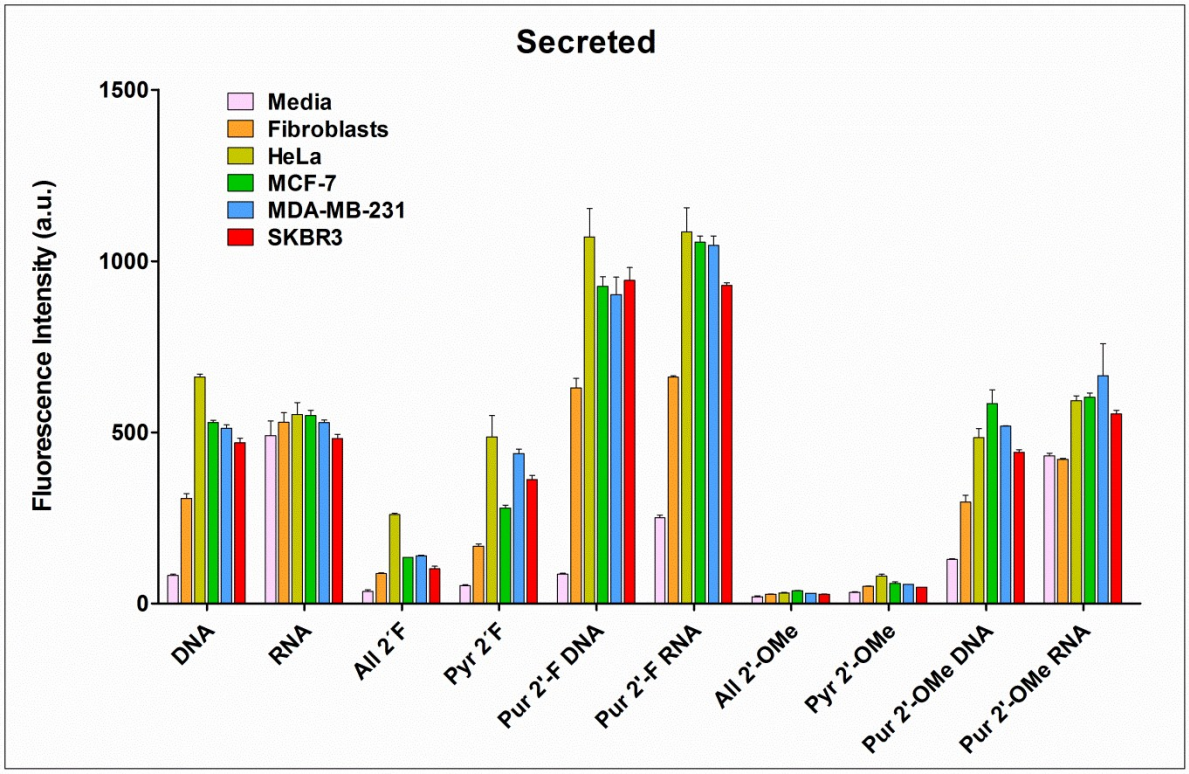

Fig S2. Evaluation of the secreted nuclease activity of healthy and cancer cells. Grouped bars show the activation profile of various nucleic acid probes (see Table S1 for probe sequence) by 48h culture media from various cancer cells lines and healthy cells control (Fibroblasts). Cancer secreted nuclease activity is reported as fluorescence intensity using various oligonucleotide probes. The bars represent the mean ± s.d. of triplicate fluorescence measurements. Data is representative of at least 3 individual experiments.

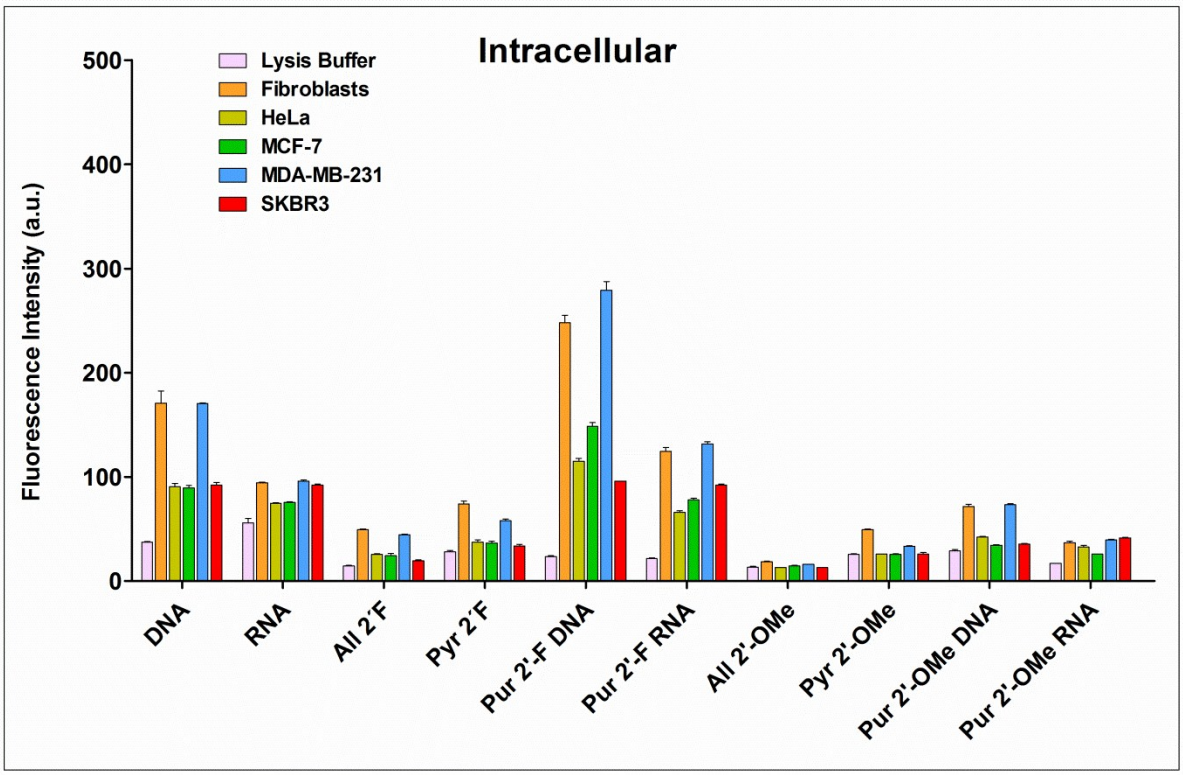

Fig S3. Evaluation of intracellular nuclease activity of healthy and cancer cells. Cell lysates obtained from different cancer cell types (HeLA, MCF-7, MDA-MB-231, SKBR3) or healthy cells (Fibroblasts), were assayed for the nuclease activity**.** Cancer intracellular nuclease activity is reported as fluorescence intensity using various oligonucleotide probes. Fibroblast lysates were used as negative control for the intracellular nuclease activity and the Lysis Buffer was used as the background control for the oligonucleotide probes. The bars represent the mean ± s.d. of triplicate fluorescence measurements. Data is representative of at least 3 individual experiments.

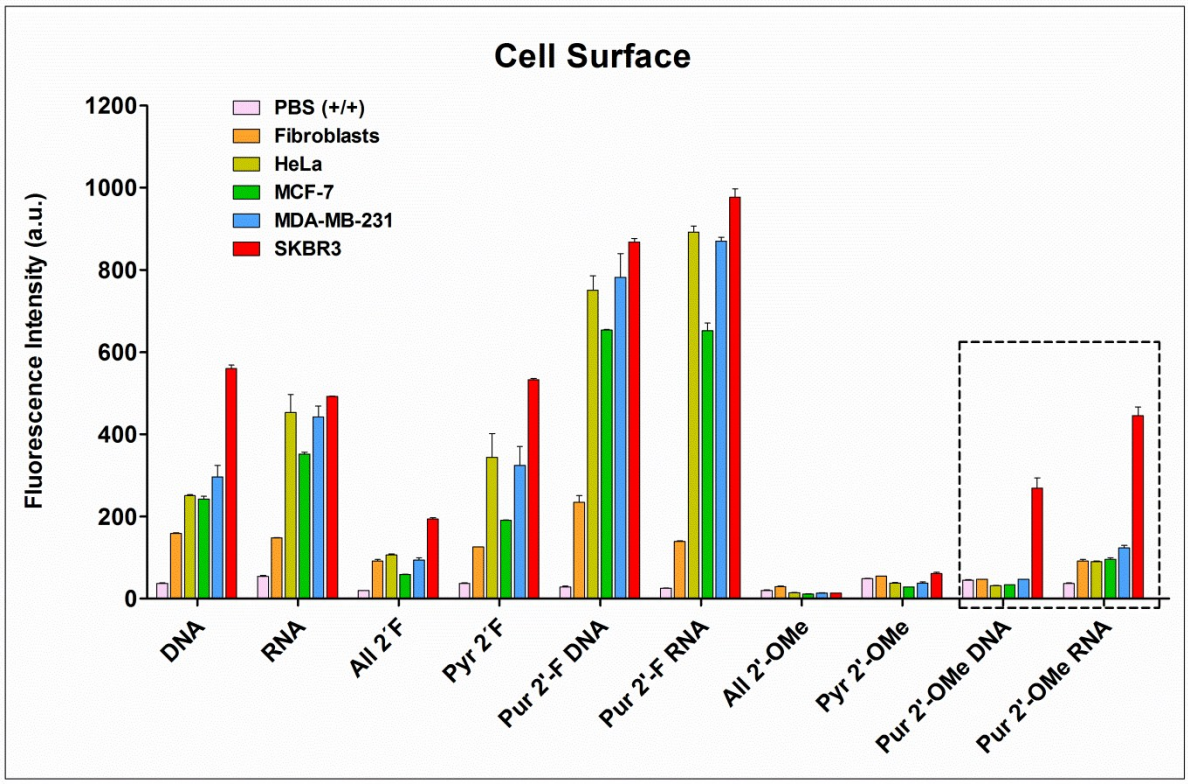

Fig S4. Evaluation of cell surface nuclease activity of healthy and cancer cells. Cell suspensions of different cancer cells (HeLA, MCF-7, MDA-MB-231, SKBR3) or healthy cells (Fibroblasts), were assayed for the nuclease activity. Cancer cell surface associated nuclease activity is reported as fluorescence intensity using various oligonucleotide probes (see Table S1 for details). High fluorescence signal represents high efficiency of the nucleases to degrade nucleic acids substrates containing various sequences and chemistries. The bars represent the mean ± s.d. of triplicate fluorescence measurements. Data is representative of at least 3 individual experiments.

MATERIALS AND METHODS

1. Materials

Ethylenediaminetetraacetic acid pH 8.0 (EDTA, CAS number: 60-00-4), Tris-EDTA, 1x Solution (pH 8) (CAS number : 38641-82-6), Nitrotriacetic acid (NTA, CAS number: 139-13-9) were purchased from ThermoFisher Scientific, Madrid, Spain. Ethylene glycol-bis(2-aminoethylether)-N (EGTA, CAS number: 67-42-5), Calcium Chloride (CAS number: 1043-52-4), Magnesium chloride hexahydrate (CAS number: 7791-18-6), Copper (II) acetate (CAS number: 142-71-2), Manganese (II) chloride (CAS number: 7773-01-5), Zinc chloride (CAS number: 7646-85-7) were purchased from Sigma- Aldrich, Madrid, Spain. Oligonucleotide probes were synthesized and purified at Biomers.net GmbH, Ulm (Germany). The oligonucleotide sequences used in this study are given above in Table S1.

2. Methods

Oligonucleotide probe synthesis and purification

Oligonucleotide probes were synthesized and purified at Biomers.net (Germany). Briefly, all the probes were synthesized with the FAM fluorophore at the 5'-end and the quencher TQ2 at the 3'-end using standard solid-phase phosphoramidite chemistry, followed by high-performance liquid chromatography (HPLC) purification. The probe identities were confirmed with matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). The purity of the probes, as assessed with HPLC analysis, is typically greater than 95%.

Cell cultures

Healthy cells (Fibroblasts), cervical cancer cells (HeLa) and breast cancer cells (MCF-7, MDA-MB-231, SKBR3) were obtained from Istanbul Kemerburgaz University (Dr. Cengiz Ozalp). All cells were cultured in Dulbecco's Modified Eagle Medium (Gibco ® DMEM, Fisher Scientific) supplemented with 10% FBS and 1% Penicillin/Streptomycin solution (LOT# 1688253, Fisher Scientific), in a humidified incubator at 37°C and 5% CO2. Cells were grown in 100 mm tissue culture dishes (Corning Fisher) until 80% confluent. Cells were further processed depending on the type of assay performed.
For the cell surface nuclease activity assay, cells were first washed twice with 5 ml PBS (+/+; containing Ca^{2+} and Mg^{2+}) and then gently scrapped using a rubber policemen (Corning 3010 Fisher) in 2 ml of PBS (+/+). Next, cells viability and counts were determined using an automatic counter (Countess II; Life Technologies). Cell counts were normalized to $1.5x10^6$ cells/ml for all cell lines and this cell concentration was used throughout all experiments reported herein, otherwise specified.
For the intracellular nuclease activity assay, cells were first washed with 5 ml of ice cold PBS (-/-; without Ca^{2+} and Mg^{2+}) to remove any traces of FBS. Cells were then harvested with 1 ml of 0.05% Trypsin /EDTA (Fisher) and further washed twice with 1 ml ice cold PBS(-/-). Subsequently, the cell pellets were resuspended in 1 ml ice cold Lysis Buffer (Tris-HCl, pH 8 + 1% Triton X-100) containing 10 µl/ml Protease Inhibitor Cocktail, EDTA free (LOT# QI20730910). Cells were allowed to stand on ice for 30 min and vortexed for 10-15 s every 10 min. Finally, the resulting cell lysate mixtures were centrifuged at 14000xg for 15 min at 4°C. Supernatant containing the intracellular phase was transferred to a new 1.5 Eppendorf tube and either assayed immediately for nuclease activity or stored at -80°C for longer term.

For the secreted nuclease activity, cells were plated in 6 well plates (in duplicates), at a density of $0.3x10^6$ cells/ml and 3ml of phenol-free DMEM media, containing 10% inactivated FBS and 1% Penicillin/Streptomycin. The culture media containing secreted nucleases was harvested after 48h and centrifuged to pellet any floating cells. Subsequently, the conditioned media was used to perform the nuclease activity assay.

Nuclease activity assay

Nuclease activity assay was performed as previously reported (Hernandez *et al.* Nucleic Acid Therapeutics 2012). Specifically, for each reaction, 9 µl of sample (e.g control buffer, cell suspension, cell lysates, condition media) were combined with 1µL (50pmoles) of oligonucleotide probe (nuclease substrate) and incubated at 37°C for 1 h. After the incubation period, the reaction was stopped by adding 295µL of PBS (-/-) supplemented with 10mM EDTA. Next, 95 µL of each sample was loaded in triplicates into a 96well plate (96F non-treated black microwell plate, Thermo Scientific). Fluorescence intensity was measured with a fluorescence microplate reader (Synergy HT, BioTek) using the filter settings for FAM (excitation/emission (ex/em), 494/521 nm)).

Conditions for the Nuclease activity

Chemical conditions: SKBR3 cells were grown as described. Cells were washed twice with PBS (+/+) and harvested by scrapping. Cells were counted and concentration adjusted to $1.5x10^6$ cells/ml. Next, 8 µl of cell suspensions were mixed with either 1µl of 50mM chelators: EDTA, EGTA and NTA or 1 µl of 50mM divalent cations: Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+}. Furthermore, each 9 µl of reaction mixture or 9 µl of cells only (in PBS+/+ and PBS-/-) were combined with 1µL (50 pmoles) of oligonucleotide probes (All 2'-OMe, Pur 2'-OMe DNA and Pur 2'-OMe RNA) and incubated at 37°C for 1 h. The reaction was stopped by adding 295 µL of PBS (-/-) supplemented with 10 mM EDTA. Next, 95 µL of each sample was loaded in triplicates into a 96 well plate and fluorescence intensity read by a plate reader, as previously described.

Temperature conditions: Cells were prepared as described above and 50 µl of cell suspensions were incubated for 30 min at 37, 65 and 95°C, respectively. Subsequently, nuclease activity assay was carried out as described, by using 9 µl of temperature treated cell suspension and 1µl of probe (All 2'-OMe, Pur 2'-OMe DNA and Pur 2'-OMe RNA).

Trypsin treatment

Fibroblasts and SKBR3 cells were grown as described above, until 80% confluent. Cells were washed twice with 5 ml PBS (-/-) and gently scrapped with a rubber policemen in a final volume of 2 ml PBS (-/-). Cells were further counted, normalized and 80 µl of equal number of cells of each type (SKBR3 and Fibroblasts) were transferred to Eppendorf tubes. Next, cells were pelleted by spinning them at 11000xg for 1 min. Pellets were further resuspended in 100 µL of 2-fold serial dilutions of Trypsin in PBS (-/-) (100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56% and 0% Trypsin) and incubated at 37°C for 1, 5 and 10 min. When incubation time elapsed, trypsin was quickly inactivated by adding 200 µl of FBS containing cell media (DMEM) to each tube. Cells were pelleted as described above, washed twice with 500 µl PBS (+/+), and resuspended in 20 µl PBS (+/+). Immediately, nuclease activity assay was carried out.