Molecular MRI of the estrogen receptor in human breast cancer cells

A. Pais[1], C. Gunanathan[2], D. Milstein[2], and H. Degani[1]

[1]Biological Regulation, Weizmann Institute of Science, Rehovot, Israel, [2]Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel

Purpose: To develop a non-invasive MRI method for the detection and quantification of estrogen receptor (ER) level *in vitro* and *in vivo*, and further using this method to investigate ER expression and degradation and to detect metastasis of ER+ cells.

Introduction: Estrogens are steroids that function as the primary sex hormones in females, regulating an assortment of activity in the female reproductive organs and the development and function of the mammary gland. Estrogens enter freely the cells and bind to nuclear ER, which act as potent transcription factor involved in differentiation and proliferation of cells. The stimulation of cell division has a key role in the development and progression of a large proportion of breast cancers. Evaluation of the ER level in breast cancer is a major and well-established tool for assessment of prognosis and for predicting response to endocrine therapy with antiestrogens such as tamoxifen. The levels of ERα in human breast cancer biopsies span a broad range reaching 1000 fmol/mg cytosol protein. However, the current clinical methods for determining ERα are semiquantitative and suffer from technical difficulties and analytical variations which may lead to inconsistent results. Furthermore, these techniques measure the level in a small fraction of the tumor, which may not reflect the actual distribution in the entire tumor. Part of these limitations can be overcome by using a non-invasive molecular imaging technique that will specifically quantify the level and distribution of ER over the whole tumor.

Methods: For targeting the ER, two novel contrast agents, composed of a gadolinuim-pyridiniumtetraacetic acid conjugated to 17β-estradiol, EPTA-Gd (1), or to tamoxifen, TPTA-Gd, (Fig.1) were synthesized. The corresponding europium (Eu)-chelates were also synthesized to act as reporters in fluorescence microscopy. Binding affinities to ER were determined by competitive radiometric binding assays using tritiated 17β-estradiol and human recombinant ER. T1 and T2 relaxivities of water protons were measured in solution using a 4.7 Tesla Biospec spectrometer (Bruker, Germany) employing standard protocols. The cellular hormonal-induced activities of the novel contrast agents were tested using the MTT assay in ER-positive and negative human breast cancer cells. Determination of ER and c-myc induced levels were assessed by western blot analysis using anti-ER and anti-c-myc antibodies, respectively. MRI studies: T1 and T2 relaxivity measurements were performed on MDA-MB-231 human breast cancer cells engineered to express ERα under tetracycline and cultivated on Biosilon beads (Fig.2) using low and high resolution MRI. In the low resolution MRI experiments ER+ and ER– cells were treated for ~ 1h with ~5 uM of the novel contrast agents - and then were washed with estrogen-free medium. The cell samples were placed in well plates. Images were recorded on a 4.7 Tesla Biospec spectrometer (Bruker, Germany). T1 relaxation rates were measured using spin echo varying TRs. T1 values were calculated applying two parameters non linear least square fit. In the high spatial resolution data (130 × 50 × 67 u^3) the cells were placed in a 5mm NMR tube and kept at 15 ^{0}C. Images were recorded on a 9.4 T DMX spectrometer (Bruker, Germany) equipped with a micro-imaging probe. Region of interest of cells was selected on a proton density weighted image excluding from the analysis regions of beads void of signal. Fluorescence studies: The Cells on beads were incubated with the novel Eu-contrast agents. Extensive washing of the cells was followed by addition of enhancement solution (PerkinElmer), and scanning the cells by time-resolved fluorescence (TRF) on a wallac VICTOR instrument (340nm excitation, 400us delay, and emission collection at 615nm).

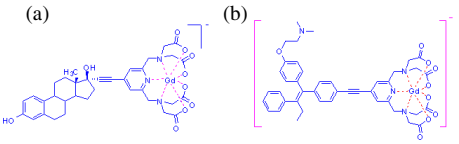

Figure 1. Schematic presentation of EPTA-Gd (a) and TPTA-Gd (b).

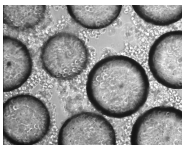

Figure 2. Light microscope image of MDA-MB-231 cells seeded on agarose beads.

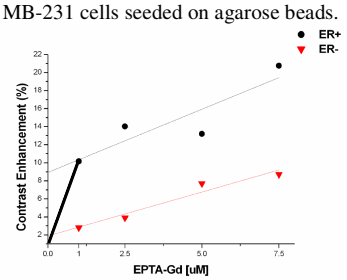

Figure 3. Contrast enhancement in ER+ and ER- MDA-MB-231 human breast cancer cells as a function of the concentration EPTA-Gd using a T_1 weighted spin echo sequence.

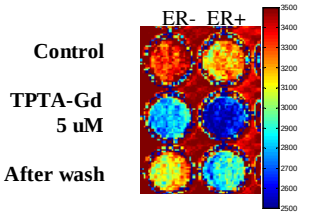

Figure 4. T1 maps of ER+ and ER- MDA-MB-231 cells cultivated on Biosilon beads using standard medium, in the presence of 5 uM TPTA-Gd, and after washout of TPTA-Gd with standard medium.

Results: ***Biological studies*****:** EPTA-Gd showed a stimulating growth effect with time- and dose-response in two ER+ cell lines similar to that of E2. TPTA-Gd, although based on the antiestrogen tamoxifen, demonstrated only a weak agonistic effect. In addition, EPTA-Gd, like E2, induced ER degradation in these cells, albeit at a slow rate, indicating their binding to ER, whereas TPTA-Gd had no effect on ER level, in a similar manner to that of Tamoxifen. Moreover, western blot analysis demonstrated that both EPTA-Gd and TPTA-Gd stimulate the expression of the proto-oncogene c-myc in ER+ cells with the effect of EPTA-Gd more pronounced.

MRI studies**:** Macroscopic enhancement and T_1 measurements of the cell systems in the presence of increasing concentrations of EPTA-Gd indicated higher enhancement in ER+ cells as compared to the ER- control cells (Fig,3). The changes with concentration represent the enhancement due to both free and ER bound agents. In ER- cells most of EPTA-Gd is free in solution, in ER+ cells part of it is bound to ER and the bound complex is expected to have a much higher relaxivity due to the high MW (slow tumbling rate). The bold line to (0,0) of ER+ cells demonstrates the effect of the predicted high relaxivity of EPTA-Gd bound to the ER (assuming full binding at ~1μM). Macroscopic T_1 measurements in the presence and absence of TPTA-Gd (5 uM) and after washout of TPTA-Gd indicated higher enhancement and difference in T1, between TPTA-Gd-treated ER+ cells and the control ER+ cells as compared to the TPTA-Gd-treated ER- cells and the ER- control cells, specifically after removal of the free and nonbound TPTA-Gd from the cell system by washing with standard medium (Fig.4). T2 measurements (data not shown) indicated significant differences as well after washout of the contrast agent in comparison to control TPTA-Gd free cells. Similar experiment performed at high resolution confirmed the results obtained at low resolution.

Fluorescence studies**:** Preliminary TRF measurements of EPTA-Eu in the same cell system have revealed specific binding of EPTA-Eu to ER+ cells as compared to ER- cells. Direct microscopic studies of both EPTA-Eu and TPTA-Eu are underway.

Conclusion: The first selective estrogen receptor modulators conjugated to Gd(III) contrast agents, EPTA-Gd, and TPTA-Gd were tested for their binding to ER, biological activity in human breast cancer cells and MRI relaxivity properties in solution and living breast cancer cells. Both showed affinities to ER at uM concentrations and agonistic effect on cell proliferation but disparity in inducing ER degradation. These novel contrast agents displayed in solution a twofold higher T1 relaxivity than the common Gd-based contrast agents and several fold higher T2 relaxivity. Increased T1 and T2 relaxation rates in viable ER+ cells cultivated on beads as compared to these rates in the same cells with null ER and concentration dependent studies indicated a specific enhancement due to their binding to ER which reflected the level of this receptor.

References 1. Gunanathan C et al., Bioconjug Chem. 2007 Sep-Oct;18(5):1361-5