Modulation of oestrogen action by receptor gene inhibition

T.A. Madden *, D. Barrow, R.A. McClelland, J.M.W. Gee, R.I. Nicholson

Tenovus Cancer Research Centre, Welsh School of Pharmacy, Redwood Building, King Edward VII Ave, Cardiff CF10 3XE, UK

Abstract

Selective oestrogen receptor downregulators (SERDs) are a class of highly effective steroidal antitumour agents that reduce cellular levels of the oestrogen receptor (ER). In this study, we compared the efficacy by which three novel molecular approaches: (1) antisense oligonucleotides; (2) antisense RNA; and (3) dominant negative mutants are able to act as SERDs. Using transient and, where appropriate, stable gene transfection experiments we found that constitutive overexpression of ER antisense RNA and a hormone-binding domain compromised dominant-negative ER mutant (*DNER-1*), were most effective at downregulating ER expression and/or activity *in vitro*.

Keywords: Breast cancer; Oestrogen receptor; Antisense; Dominant-negative; Gene inhibition

1. Introduction

Selective downregulation of oestrogen receptor (ER) expression has recently been recognised as a property of the new steroidal, pure-anti-oestrogen Faslodex™ (FAS). As FAS appears to be more effective than tamoxifen at reducing both ER expression and proliferation of primary breast cancers [1], there is much interest in determining if this is responsible for its increased antitumour efficacy. Thus, we have attempted to create a cell culture model which mimics the selective loss of ER expression and/or activity observed following FAS treatment, by comparing the relative efficacy with which three different novel ER gene inhibition strategies are able to act as SERDs *in vitro*.

2. Materials and methods

2.1. Antisense oligonucleotides

Two chimeric methylphosphonate-phosphodiester antisense oligonucleotides [2] were synthesised (Oswell DNA Services, UK), a 15mer antisense ODN targeting the ER translation start codon (5′ C*A*T*G-G-T-C-A-T-G-G-T*C*A*G) and a scrambled arrangement of bases present in the antisense ODN (5′A*T*C*G-T-G-G-A-T-C-G-T*G*A*C) (*methylphosphonate-phosphodiester internucleoside linkage).

2.2. Expression vectors

For ER transcription activation studies, the oestrogen-responsive plasmid ERE-*Tk-Luc* [3] was employed. Full length and truncated ER antisense RNA expression vectors (pCDNA1.1ASER and pCDNA1.8ASER) were constructed by cloning cDNA fragments from the wild type ER cDNA (HEGO) [4] into the eukaryotic expression vector pCDNA3 (Invitrogen). The dominant-negative ER mutant expression vector pCDNA*DNER-1* was constructed by deleting a 914bp Xba1 fragment from the COOH terminus of HEGO and cloning it into pCDNA3.

2.3. Cell culture and transfection

ER-negative COS-7 cells and ER-positive MCF-7 cells were routinely maintained in Roswell Park Memorial Institute (RPMI) + 10% fetal calf serum (FCS). For transient transfection cells were plated at 5×10^5 cells/cm^2 in phenol red free RPMI + 5% charcoal stripped FCS. MCF-7 transfections were performed in phenol red/serum free medium DCCM-1 using the cationic lipid Lipofectin (Gibco-BRL) including 1% dimethysulphoxide (DMSO) within the transfection mix to improve transfection efficiency. COS-7 cells were transfected as above using the cationic lipid Lipofectamine (Gibco-BRL) but excluding DMSO. MCF-7 stable transfectants were selected in RPMI + 10% FCS + 800 μg/ml of geneticin (Sigma, Poole, Dorset, UK). Resistant colonies, visible within 2 weeks were isolated using cloning cylinders (Sigma) and expanded.

2.4. ER Northern blot/immunoblot

ER protein and mRNA levels were estimated using whole cell lysates and total cellular RNA preparations

* Corresponding author. Tel.: +44-02920-875226; fax: +44-02920-875152.

E-mail address: maddenta1@cardiff.ac.uk (T.A. Madden).

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using an internal *β-Actin* gene standard as previously described [5].

2.5. Reporter gene assays

ER transactivation was determined 36 h post-transfection by measuring ERE-Tk-Firefly luciferase activity against an internal TK- Renilla-luciferase standard using a commercial assay (Promega, Southampton, UK).

2.6. Cell proliferation studies

Cell proliferation responses to steroids and antisense ODNs were evaluated over 7–14 days by direct counting of viable cells [6]. All treatments were replenished every 2 days.

3. Results

Chimeric MP-PO antisense oligonucleotides at concentrations of 0.1–1 μM had limited efficacy, reducing ER protein expression and transactivation in MCF-7 cells by 15–20%. At the higher concentrations (2–10 μM) required for effective ER gene inhibition, these ODNs lost specificity with both antisense and scrambled ODNs substantially reducing ER levels. At concentrations of below 10 μM these ODNs also failed to reduce MCF-7 basal growth. Constitutively expressed full length and truncated ER antisense RNAs reduced ER protein, mRNA and transactivation by 50, 70 and 25%, respectively over wild-type MCF-7 and pCDNA3 stably transfected MCF-7 controls, but had no effect on *actin* or G418-resistant gene expression. MCF-7 ER antisense stable transfectants exhibited no alterations in their steroid sensitivity when compared with wild-type or pCDNA3 stably transfected MCF-7 cells. In transient transfection experiments constitutive expression of an AF-2 compromised ER mutant (*DNER-1*) repressed ER transactivation in a dose-responsive manner by 70–80% in both MCF-7 and in wild-type ER transfected ER-negative COS-7 cells. The apparent trans-dominant effect of *DNER-1* on wild-type ER activity was not reflective of a general inhibition of gene transcription since in similar dose–response curves the mutant ER failed to block transcription from either the basic ERE deleted *Tk-Luc* reporter gene construct or a TPA response element bearing the *Tk-Luc* construct.

4. Conclusions

In contrast to antisense oligonucleotides, constitutively expressed ER antisense RNAs and ER dominant negative mutants appear feasible alternatives to current ER ligand derivatives as a means of selectively downregulating oestrogen/ER actions *in vitro*.

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Identification of women at high risk of developing endometrial cancer on tamoxifen

M. Berlière [a], G. Radikov [a], C. Galant [b], Ph. Piette [b], E. Marbaix [b], J. Donnez [a,*]

[a] *Department of Gynaecology, Catholic University of Louvain, Ave. Hippocrate, 10 — B-1200 Brussels, Belgium*
[b] *Department of Pathology, Catholic University of Louvain, Ave. Hippocrate, 10 — B-1200 Brussels, Belgium*

* Corresponding author. Tel.: +32-2-764-9502; fax: +32-2-7649507.
E-mail address: donnez@gyne.ucl.ac.be (J. Donnez).

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