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Human T47D-ERβ breast cancer cells with tetracycline-dependent ERβ expression reflect ERα/ERβ ratios in rat and human breast tissue

N.M. Evers [a,*], T.M.C. van de Klundert [b], Y.M. van Aesch [b], S. Wang [a,c], W.K. de Roos [d], A. Romano [e], L.H.J. de Haan [a], A.J. Murk [a], A.G.H. Ederveen [f], I.M.C.M. Rietjens [a], J.P. Groten [a]

[a] Division of Toxicology, Wageningen University, Tuinlaan 5, 6703 HE Wageningen, The Netherlands
[b] Women's Health Department, MSD, P.O. Box 20, 5340 BH Oss, The Netherlands
[c] Rikilt – Institute of Food Safety, Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands
[d] Department of Surgical Oncology, Gelderse Vallei Hospital, Ede, The Netherlands
[e] Department of Obstetrics and Gynecology, GROW-School for Oncology and Developmental Biology, Maastricht University Medical Centre, 6202 AZ Maastricht, The Netherlands
[f] Pharmacokinetics Pharmacodynamics & Drug Metabolism, MSD, P.O. Box 20, 5340 BH Oss, The Netherlands

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ABSTRACT

T47D-ERβ breast cancer cells with tetracycline-dependent ERβ expression and constant ERα expression can be used to investigate effects of varying ERα/ERβ ratios on estrogen-induced cellular responses. This study defines conditions at which ERα/ERβ ratios in T47D-ERβ cells best mimic ERα/ERβ ratios in breast and other estrogen-sensitive tissues in vivo in rat as well as in human.

Protein and mRNA levels of ERα and ERβ were analyzed in T47D-ERβ cells exposed to a range of tetracycline concentrations and compared to ERα and ERβ levels found in breast, prostate, and uterus from rat and human origin.

The ERα/ERβ ratio in T47D-ERβ cells exposed to >150 ng/ml tetracycline is comparable to the ratio found in rat mammary gland and in human breast tissue. The ERα/ERβ ratio of other estrogen-sensitive rat and human tissues can also be mimicked in T47D-ERβ cells. The ERα/ERβ ratio found in MCF-7 and native T47D breast cancer cell lines did not reflect ratios in analyzed rat and human tissues, which further supports the use of T47D-ERβ cells as model for estrogen-responsive tissues. Using 17β-estradiol and the T47D-ERβ cells under the conditions defined to mimic various tissues it could be demonstrated how these different tissues vary in their proliferative response.

1. Introduction

Estrogen receptors (ERs) modulate the effects of estrogens on cells and tissues (Rau et al., 2005) with ERα and ERβ being the two major ERs (Foryst-Ludwig et al., 2008). ERα and ERβ are encoded by distinct genes (Nilsson et al., 2001) and have different roles in gene regulation (Williams et al., 2008). As a consequence ERα and ERβ have differential effects on the cell cycle in various estrogen-sensitive tissues. It has been shown that ERα activation in breast and uterus enhances cell proliferation, which is necessary for growth and maintenance of tissues (Harris, 2007). ERβ has been shown to counteract the ERα mediated stimulation of cell proliferation (Bardin et al., 2004a; Sotoca et al., 2008a,b; Stossi et al., 2004; Ström et al., 2004). Hence ERα and ERβ have different roles in gene regulation and their relative level and varying ratio within tissues may influence the response towards different estrogens. When the response to estrogens by the endocrine system is deregulated, ERα activation might eventually result in tumor formation (Hartman et al., 2006; Lazennec, 2006; Monroe et al., 2005; Rau et al., 2005; Weitzmann and Pacifici, 2006), whilst ERβ activation has been shown to stimulate apoptosis (Imamov et al., 2004; Paruthiyil et al., 2004; Sotoca et al., 2011). In certain types of cancer, the ERα/ERβ ratio is increased compared to healthy tissue. This has been shown for both ER-positive breast tumors and ovarian carcinomas

Abbreviations: BPH, benign prostatic hyperplasia; cDNA, complementary deoxyribonucleic acid; DCC-FCS, dextran-coated charcoal-treated fetal calf serum; DL, detection limit; DNA, deoxyribonucleic acid; dNTP, deoxynucleotide triphosphate; DMEM, Dulbecco's modified Eagle's medium; EC_{50}, half maximal effective concentration; EDTA, ethylenediaminetetraacetic acid; ER, estrogen receptor; FCS, fetal calf serum; HE staining, hematoxylin and eosin staining; HP1, heterochromatin protein 1; IgG, immunoglobulin G; MCF-7 cells, human breast adenocarcinoma cells; M-MLV, Moloney Murine Leukemia Virus; mRNA, messenger ribonucleic acid; MSD, Merck, Sharp & Dohme; PBS, phosphate buffered saline; PVDF, polyvinylideenfluoride; qPCR, quantitative polymerase chain reaction; RT, reverse transcriptase; SDS, sodium dodecyl sulfate; T47D cells, human ductal breast epithelial tumor cells; T47D-ERβ cells, T47D human ductal breast epithelial tumor cells with tetracycline-dependent ERβ expression; T47D-ERβ-CS, T47D-ERβ calibration standard, the T47D-ERβ cells without tetracycline.

* Corresponding author. Tel.: +31 317 482756; fax: +31 317 484931.
E-mail address: Nynke.Evers@WUR.nl (N.M. Evers).

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(Bardin et al., 2004b; Leygue et al., 1998). It was shown that the increase in ERα/ERβ ratio was due to a decreased level of ERβ (Lazennec et al., 2001; Rutherford et al., 2000). This observation might be related to the antagonistic effect of ERβ mediated gene expression on cell proliferation induced by ERα activation (Sotoca et al., 2008a).

Given the different biological effects on the cell cycle resulting from differential ERα or ERβ activation, it is of interest to note that ERα and ERβ also differ in their relative and absolute tissue distribution and tissue levels (Nilsson et al., 2001). Levels of ERα and ERβ have been shown to vary in such a way that one of them is dominantly present in a specific tissue. For example, ERα is predominant in the uterus (Pearce and Jordan, 2004), while ERβ is predominant in the prostate (Enmark et al., 1997; Pearce and Jordan, 2004). The different levels of ERα and ERβ within specific tissues are expected to determine the responses of these tissues to estrogens and to estrogenic compounds, which may have different affinities for ERα and ERβ (Attia and Ederveen, 2011).

To investigate the potential impact of the ERα/ERβ ratio on estrogen induced cellular effects, Ström et al. (2004) developed the T47D-ERβ cell line. This model consists of T47D human ductal breast epithelial tumor cells with tetracycline-dependent ERβ expression (short, 485 amino acids isoform) and a constant ERα expression. When exposed to an increasing concentration of tetracycline, ERβ expression in these T47D-ERβ cells decreases and thus the ERα/ERβ ratio increases (Ström et al., 2004). In previous studies the cell model was applied to investigate the influence of various ERβ levels on cellular proliferation in response to 17β-estradiol, other estrogens and anti-estrogenic compounds (Ström et al., 2004; Sotoca et al., 2008b). In order to be able to better translate the findings reported in in vitro studies with T47D-ERβ cells with tetracycline-dependent ERβ expression towards the in vivo situation, the ERα/ERβ levels in these T47D-ERβ breast cancer cells at varying tetracycline concentrations have to be compared to levels actually occurring in breast and other estrogen responsive tissues in both experimental animals and humans to assess the physiological relevance, compared to the levels in vivo. Therefore, the aim of the present study was to define the conditions at which the ERα/ERβ ratio in the T47D-ERβ breast cancer cells with tetracycline-dependent ERβ expression would best mimic the actual ERα/ERβ ratio in rat and human breast and other estrogen-sensitive tissues (uterus and prostate) in vivo, to further support their use as models for estrogen responsive tissues. For comparison, also the MCF-7 and native T47D cell lines were investigated for their ERα/ERβ ratios to see to what extent these cell lines provide an adequate model for mimicking physiologically relevant ERα/ERβ ratios.

2. Materials and methods

2.1. Cell culture

The T47D human ductal breast epithelial tumor cell line (T47D) was purchased from the American Type Tissue Culture Collection (Manassas, VA, USA). The T47D-ERβ cell line was made and provided by Ström et al. (2004). T47D wild type and T47D-ERβ cells were cultured in a 1:1 mixture of Ham's nutrient mixture F12 and Dulbecco's modified Eagle's medium (DMEM) (Gibco, Paisley, Scotland, #31331-038) supplemented with 5% fetal calf serum (FCS) (Invitrogen, Paisley, Scotland, #10099). For the T47D-ERβ cells, 1000 ng/ml tetracycline (Sigma, Zwijndrecht, the Netherlands, #T7660) to fully inhibit ERβ expression (Sotoca et al., 2008b) was added to the medium. Sotoca et al. (2008b) reached maximal induction of an EGFP gene which is co-expressed with ERβ after 24 h of depleting the T47D-ERβ cells from tetracycline, thus suggesting that at 24 h also ERβ expression is maximal. ERα levels in T47D-ERβ cells are constant and quantification of the ERβ levels in the cells upon 48 h of cultivation in the absence of tetracycline revealed similar ERβ levels as detected at 24 h (data not shown). This supports that at the time of quantification of the ERα/ERβ ratios (24 h) the ERβ expression reached a steady state and ERα/ERβ ratios are stable. Therefore exposure to specific tetracycline concentrations was for 24 h.

MCF-7 human breast adenocarcinoma cells were purchased from the American Type Tissue Culture Collection (Manassas, VA, USA). They were cultured in a 1:1 mixture of Ham's nutrient mixture F12 and DMEM (Gibco, Paisley, Scotland, #31331-038) supplemented with 5% FCS. All cells were incubated at 37 °C and 5% CO_2 in a humidified atmosphere.

Because phenol red exerts estrogenic activity (Glover et al., 1988), at least 24 h before exposure, cells were washed three times with phosphate buffered saline (PBS) (Gibco, Paisley, Scotland, #10010-015) and transferred to phenol red-free medium (Gibco, Paisley, Scotland, #21041-025) supplemented with 5% hormone-free dextran-coated charcoal-treated fetal calf serum (DCC-FCS) (Perbio Science, Waltham, MA, USA, #SH30068.05).

2.2. Tissue collection

Estrogen responsive tissues from adult (13–16 weeks old) male and female Sprague Dawley rats were collected at the animal facility of Merck Sharp & Dohme (MSD) in Schaijk, the Netherlands. Before autopsy, animals were anesthesized and tissues were removed, placed in foil and immediately emerged in liquid nitrogen. This experiment was approved by the animal welfare committee of MSD. Estrus cycle data as determined by histological examination of vaginal smears after HE (hematoxylin and eosin) staining of the female rats, as described by Allen and Doisy (Allen and Doisy, 1923), are shown in Table 1.

Collection of human material was approved by the medical ethical committees of the respective hospitals. Human breast tissue was collected from female donors at hospital De Gelderse Vallei in Ede, the Netherlands. Human endometrium samples were obtained at Maastricht University Medical Centre in Maastricht, the Netherlands from pre-menopausal women undergoing hysterectomy for benign indications. Prostate tissue from male patients with benign prostatic hyperplasia (BPH) was collected at the Radboud University Nijmegen Medical Centre in Nijmegen, the Netherlands. Known relevant characteristics from the tissue donors are displayed in Table 2.

2.3. Protein and mRNA isolation

For protein and mRNA isolation, 80% confluent cells in 75 cm^2 cell culture flasks (Corning, #430641) were scraped in 1 ml Trizol (Invitrogen, Paisley, Scotland, #15596-018) and stored at −80 °C. Tissue samples were snap-frozen in liquid nitrogen and stored at −80 °C. Frozen tissue (50–100 mg) was homogenized in 1 ml Trizol using a mini-beadbeater (MBB-8 Cell Disrupter, Biospec products, Bartlesville, OK, USA) for breast and prostate tissue and by using a power homogenizer (PowerGen GLH 220, Omni International,

Table 1
Phase in estrus cycle of sampled rats as determined by histological examination of HE stained vaginal smears.

Sample no.	Phase
1	Diestrus
2	Metestrus
3	Proestrus
4	Metestrus
5	Diestrus