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Original Research

Identification of gene regulation patterns underlying both oestrogen- and tamoxifen-stimulated cell growth through global gene expression profiling in breast cancer cells

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Abstract ***Purpose:*** A c-Src inhibitor blocks oestrogen (E_2)-induced stress and converts E_2 responses from inducing apoptosis to growth stimulation in E_2-deprived breast cancer cells. A reprogrammed cell line, MCF-7:PF, results in a functional oestrogen receptor (ER). We addressed the question of whether the selective ER modulator 4-hydroxytamoxifen (4-OHT) could target ER to prevent E_2-stimulated growth in MCF-7:PF cells.

Methods: Expression of mRNA was measured through real-time RT-PCR. Global gene expression profile was analysed through microarray. Transcriptome profiles were screened by RNA-sequencing.

Results: Unexpectedly, both 4-OHT and E_2 stimulated cell growth in a concentration-dependent manner. Expression profiling showed a remarkable overlap in genes regulated in the same direction by E_2 and 4-OHT. Pathway enrichment analysis of the 280 genes commonly deregulated in MCF-7:PF cells by 4-OHT and E_2 revealed functions mainly related to membrane, cytoplasm and metabolic processes. Further analysis of 98 genes up-regulated by both 4-OHT and E_2 uncovered a significant enrichment in genes associated with membrane remodelling, cytoskeleton reorganisation, cytoplasmic adapter proteins, cytoplasm organelle proteins and related processes. 4-OHT was more potent than E_2 in up-regulating some membrane remodelling molecules, such as *EHD2*, *FHL2*, *HOMER3* and *RHOF*. In contrast,

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4-OHT acted as an antagonist to inhibit expression of the majority of enriched membrane-associated genes in wild-type MCF-7 cells.

Conclusions: Long-term selection pressure has changed the cell population responses to 4-OHT. Membrane-associated signalling is critical for 4-OHT-stimulated cell growth in MCF-7:PF cells. This study provides a rationale for the further investigation of target therapy for tamoxifen resistant patients.

1. Introduction

Oestrogen (E_2) plays a pivotal role in the development and progression of breast cancers. As a result, blockade of the E_2 signal through either aromatase inhibitors (AIs) or tamoxifen is important therapeutic strategy to treat or prevent oestrogen receptor (ER) positive breast cancers [1]. However, the acquired resistance to anti-oestrogen therapies is still a challenge in the clinic. Laboratory findings that re-transplantation of tamoxifen-stimulated MCF-7 tumours into successive generations of athymic mice over 5 years results in the selection of a resistant tumour cell population that is killed by physiological levels of E_2 [2,3], has resulted in the new biology of E_2-induced apoptosis [4–6]. Indeed, E_2-induced apoptosis has been used to treat breast cancer after failure of AI therapy [7] and used to explain the action of E_2 replacement therapy for postmenopausal women in their 60s having a lower incidence of breast cancer and mortality [8]. All of these clinical relevant findings encouraged us to investigate the mechanisms underlying E_2-induced apoptosis in long-term E_2-deprived breast cancer.

The function of oncogene c-Src is closely associated with the ER and has been reported to mediate resistance to endocrine therapy in breast cancer cells [9,10]. Our previous findings show that c-Src activity is increased in two long-term E_2-deprived breast cancer cells [11], which mediates the non-genomic and genomic pathways of E_2 [12]. In addition to the role of c-Src in growth, the oncogene participates in stress responses induced by E_2 [12], which are critical to induce apoptosis [12–14]. Inhibition of c-Src blocks E_2-induced apoptosis [12,15]. Unexpectedly, a resulting cell line, MCF-7:PF, is established when treatment is extended for 2 months to mimic clinical trials [15]. Rather than undergoing apoptosis a new E_2-stimulated population emerged [15]. It raises the question whether the antioestrogen 4-hydroxytamoxifen (4-OHT) can block the E_2-stimulated growth in MCF-7:PF cells.

In this study, we found that 4-OHT stimulated MCF-7:PF cell growth in an ER-dependent manner and could not block E_2-stimulated growth. Global gene expression microarray revealed a remarkable overlap in genes deregulated in the same direction by E_2 and 4-OHT in MCF-7:PF cells, which could be blocked by the pure antioestrogen ICI 182,780 (ICI). Pathway enrichment analysis of the 280 genes commonly deregulated by 4-OHT and E_2 revealed the top 13 functions are mainly related to membrane, cytoplasm and metabolic processes. Together, these results suggest that membrane-associated signalling may be involved in the tamoxifen-stimulated growth.

2. Materials and methods

2.1. Materials

Estradiol (E_2) and 4-OHT were purchased from Sigma–Aldrich (St. Louis, MO); ICI 182,780 (ICI) was purchased from Tocris (Park Ellisville, MO). c-Src inhibitor, PP2 and IGF-1Rβ inhibitor, AG1024, were purchased from CalBiochem (San Diego, CA).

2.2. Cell culture conditions and cell proliferation assays

The ER-positive wild-type human breast cancer MCF-7 cells, oestrogen-deprived MCF-7:5C cells and MCF-7:PF cells were cultured as previously described [15]. The DNA fingerprinting pattern of all cell lines is consistent with the report by the ATCC [15]. The DNA content of the cells, a measure of proliferation, was determined as previously described [15] using a DNA fluorescence Quantitation kit (Bio-Rad Laboratories, Hercules, CA).

2.3. Quantitative real-time RT-PCR

Quantitative real-time RT-PCR assays were conducted as previously described [12] using the SYBR Green PCR Master Mix from Applied Biosystems (Foster City, CA) and a 7900HT Fast Real-time PCR System (Applied Biosystems). All primers were synthesised by Integrated DNA Technologies (San Diego, CA).

2.4. RNA sequencing (RNA-seq) analysis

In brief, wild-type MCF-7 cells were treated with vehicle control (0.1% ethanol), E_2 (10^{-9} mol/L) and 4-OHT (10^{-6} mol/L) for 24 h. Total RNA was isolated with an RNeasy Micro kit (Qiagen, Valencia, CA). These RNA samples were first converted into a library of cDNA fragments. Sequencing adaptors were subsequently added to each cDNA fragment and a