MOLECULAR ONCOLOGY 9 (2015) 1744–1759

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Tamoxifen induces a pluripotency signature in breast cancer cells and human tumors

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ARTICLE INFO

Article history:
Received 6 May 2015
Accepted 20 May 2015
Available online 5 June 2015

Keywords:
Breast cancer
Tamoxifen
Pluripotency
Endocrine resistance
Transcriptomic analysis
Cell lines
Patient data

ABSTRACT

Tamoxifen is the treatment of choice in estrogen receptor alpha breast cancer patients that are eligible for adjuvant endocrine therapy. However, ~50% of ERα-positive tumors exhibit intrinsic or rapidly acquire resistance to endocrine treatment. Unfortunately, prediction of *de novo* resistance to endocrine therapy and/or assessment of relapse likelihood remain difficult. While several mechanisms regulating the acquisition and the maintenance of endocrine resistance have been reported, there are several aspects of this phenomenon that need to be further elucidated. Altered metabolic fate of tamoxifen within patients and emergence of tamoxifen-resistant clones, driven by evolution of the disease phenotype during treatment, appear as the most compelling hypotheses so far. In addition, tamoxifen was reported to induce pluripotency in breast cancer cell lines, in vitro. In this context, we have performed a whole transcriptome analysis of an ERα-positive (T47D) and a triple-negative breast cancer cell line (MDA-MB-231), exposed to tamoxifen for a short time frame (hours), in order to identify how early pluripotency-related effects of tamoxifen may occur. Our ultimate goal was to identify whether the transcriptional actions of tamoxifen related to induction of pluripotency are mediated through specific ER-dependent or independent mechanisms. We report that even as early as 3 hours after the exposure of breast cancer cells to tamoxifen, a subset of ERα-dependent genes associated with developmental processes and pluripotency are induced and this is accompanied by specific phenotypic changes (expression of pluripotency-related proteins). Furthermore

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http://dx.doi.org/10.1016/j.molonc.2015.05.008

we report an association between the increased expression of pluripotency-related genes in ERα-positive breast cancer tissues samples and disease relapse after tamoxifen therapy. Finally we describe that in a small group of ERα-positive breast cancer patients, with disease relapse after surgery and tamoxifen treatment, ALDH1A1 (a marker of pluripotency in epithelial cancers which is absent in normal breast tissue) is increased in relapsing tumors, with a concurrent modification of its intra-cellular localization. Our data could be of value in the discrimination of patients susceptible to develop tamoxifen resistance and in the selection of optimized patient-tailored therapies.

1. Introduction

The discovery of estrogen receptors (ERs) (Jensen et al., 1967a, 1967b) and the introduction of selective estrogen receptor modulators (SERMs) after the early '70s, with tamoxifen as the leading molecule, have revolutionized our concept of targeted and personalized therapy for the treatment of breast cancer patients (Jensen and Jordan, 2003; Jordan, 1999, 2001c, 2006). Tamoxifen has been shown to be highly effective in ERα-positive patients (Early Breast Cancer Trialists' Collaborative, 1998, 2005), being the gold standard therapy for this group. Indeed, tamoxifen adjuvant therapy for five or more years leads to significant enhancement of disease-free or overall survival; however, about 50% of patients develop secondary resistance to endocrine therapy, experience relapse and subsequently die from the disease (Early Breast Cancer Trialists' Collaborative, 1998, 2005), although, in ERα-positive patients, tamoxifen may provide a very long period of protection, even after the cessation of therapy (Cuzick et al., 2015). Tamoxifen resistance has been attributed to either a variability of tamoxifen metabolic fate by individual patients, concurring by the identification of low, normal and increased tamoxifen metabolizers (Dehal and Kupfer, 1997; Goetz et al., 2005), or to the evolution of the disease phenotype during treatment, with the emergence of tamoxifen-resistant clones.

Some reports, related to failure of tamoxifen treatment in breast cancer, have identified that long term exposure to the drug may induce the emergence of tamoxifen-resistant ERα-positive cell clones, on which estrogen may induce apoptosis (Ariazi et al., 2011) and the emergence of a stem cell-like population of resistant cells in cell lines (Ao et al., 2011; Iliopoulos et al., 2009) or cells deriving from primary tumors (Kok et al., 2009; Manni et al., 1985). Additionally, it was reported that tamoxifen-resistance may originate from cell populations within the cell culture with stem cell characteristics (Lin et al., 2013; Liu et al., 2013; Martinez-Outschoorn et al., 2011). However, the time-frame needed for tamoxifen to achieve this cell transformation is yet unclear. Interestingly, in addition to ER-related effects, tamoxifen has been reported to exert ER-unrelated actions (Frasor et al., 2006), such as effects on body weight, bone growth, pituitary function (Fitts et al., 2011) and induction of cell cycle arrest and apoptosis (Morad et al., 2013). Therefore, the induction of pluripotency by the drug may also be mediated through similar, ER-unrelated actions.

A large number of studies (~1700 hits on Medline) have dealt with transcriptional changes induced by tamoxifen (see Dimitrakopoulou et al., 2014; Fan et al., 2014; Frasor et al., 2006; Lebedeva et al., 2012; Oyama et al., 2011, for specific examples in breast cancer cells). However, none of the aforementioned studies has described a specific hint on early tamoxifen-induced pluripotency. In view of the above and in order to identify how early pluripotency-related effects of tamoxifen may occur, we have performed a whole transcriptome analysis of an ER-positive and a triple-negative breast cancer cell line, shortly exposed to tamoxifen (3 h), in order to decipher direct actions of the agent on the transcriptome. Our specific aim was to identify whether early/direct tamoxifen transcriptional actions related to induction of pluripotency, were mediated through specific ERα-dependent, or ER-unrelated transcription. Our data show that early exposure to tamoxifen triggers modifications of an ERα-dependent subset of genes, related to developmental processes and pluripotency. Furthermore we report an association between the level of expression of pluripotency-related genes in ERα positive breast cancer tissues (prior to treatment) and disease relapse after tamoxifen administration, in a retrospective cohort of patients. Finally, in a small group of ERα-positive breast cancer human tumors with disease relapse after surgery and tamoxifen treatment, we identified an increase of ALDH1A1 expression and a modification of its intracellular distribution.

2. Material and methods

2.1. Cell lines

The human breast cancer cell lines T47D and MDA-MB-231 were obtained from DSMZ (Braunschweig, Germany) and were cultured in RPMI 1640, supplemented with 10% fetal bovine serum, at 37 °C, with 5% CO_2. All chemicals were purchased from Sigma (St Louis, MO), unless stated otherwise.

2.2. Whole transcriptome assay and analysis

After a 4 h incubation in a medium containing 10% charcoal stripped FBS, cells were incubated in the same conditions,