EXPERT REVIEWS

Predicting response: identifying molecular determinants of endocrine response and resistance in breast cancer

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Evaluation of: Fernandez-Cuesta L, Anaganti S, Hainaut P, Olivier M. p53 status influences response to tamoxifen but not to fulvestrant in breast cancer cell lines. *Int. J. Cancer* 128, 1813–1821 (2011).

Blocking estrogen activity or production through the use of anti-estrogens such as tamoxifen and aromatase inhibitors, respectively, has had a significant impact on improving the survival of breast cancer patients. However, innate or acquired resistance to these endocrine therapies remains a major clinical problem and a challenge to the successful treatment of this disease. A recent article explored the role of the tumor-suppressor gene *TP53* in the response of breast cancer cell lines to tamoxifen and the pure anti-estrogen, fulvestrant. Mutations in *TP53*, which occur frequently in breast cancer like many other types of neoplasia, are already known to negatively influence prognosis, but here their role in the response to anti-estrogen treatment was evaluated. This study found that cells harboring p53 mutations were more resistant to the cytotoxic effects of 4-hydroxytamoxifen than their p53 wild-type counterparts. Furthermore, mutant p53 cells were actually stimulated by low concentrations of 4-hydroxytamoxifen, with evidence that this may be mediated through enhanced growth factor signaling. By contrast, p53 status did not affect the response to fulvestrant. This article further delineates the role of p53 as a determinant of the endocrine response.

Keywords: anti-estrogen • breast cancer • EGFR • fulvestrant • HER2 • p53 • tamoxifen

Understanding the molecular basis of anti-estrogen resistance and developing therapeutic strategies to combat it are major priorities in improving the survival of breast cancer patients. Mutations in the *TP53* gene are the most prevalent in human cancers and are associated with poor prognosis in breast cancer – most probably owing to p53's key roles in the regulation of proliferation and survival in response to chemotherapeutic challenge. In the paper under evaluation, the role of mutations in p53 in the response of breast cancer cells to the selective estrogen receptor modulator tamoxifen (TAM) and the pure anti-estrogen fulvestrant was explored [1].

Summary of methods & results

This study was carried out in four estrogen receptor (ER)-positive human breast cancer cell lines with differing p53 status: ZR-75-1 have wild-type (WT) p53; BT-474 carry a temperature-sensitive mutation at E285K that adopts a WT conformation at 32°C but has a mutant (MUT) conformation at 37°C; and MN1 and MDD2 are isogenic cell lines derived from MCF-7 cells that have WT and MUT p53 status, respectively. MDD2 cells are stably transfected with a p53 mini-protein (containing the first 14 and last 89 amino acids of mouse p53) that exerts a dominant negative effect, inactivating the endogenous WTp53 protein.

In order to determine the effects of p53 status on the cellular response to estrogen and anti-estrogens, the cell lines were treated with increasing concentrations of an active metabolite of TAM, 4-hydroxytamoxifen (4-OHT), fulvestrant or 17β-estradiol, and the effects on both

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proliferation and cell survival were determined by MTS assay. The most striking results were observed in the isogenic cell lines, where the WTp53 MN1 cells exhibited a cytotoxic response to 4-OHT even at low concentrations (<1 μM), while their MUTp53 counterparts (MDD2) appeared to be growth stimulated at all concentrations up to 10 μM. A similar p53-dependent differential response was observed between ZR-75-1 (WTp53) and BT-474 (MUTp53) cells, although the responses were less marked. In support of these observations, silencing of WTp53 function in both MN1 and ZR-75-1 cells using p53-specific siRNA led to a 4-OHT response that mimicked that observed in the MUTp53 cells; namely, abrogation of 4-OHT-mediated cytotoxicity and proproliferative effects at low concentrations. MUTp53 expression was also associated with hypersensitivity to the proliferative effects of estradiol compared with WTp53-expressing cell lines. By contrast, treatment with fulvestrant produced a cytotoxic response (albeit to varying degrees) in all of the cell lines, regardless of their p53 status.

To further explore the molecular mechanisms underlying this p53-dependent TAM response, the expression of known p53 target genes involved in cell cycle regulation (*SFN/14-3-3* and *p21*), DNA repair (*GADD45*) and apoptosis (*BBC3*, *BCL2* and *BAX*), was determined in response to 4-OHT treatment in MN1 and MDD2 cells. The results revealed that 4-OHT did not elicit a classical p53 response; while p21 protein was induced by high concentrations of 4-OHT (10 μM) in a WTp53-dependent manner as expected, only *GADD45* and *BCL-2* were transcriptionally modulated following treatment and this was observed in both WT and MUT p53-expressing cells. Furthermore, at 1 μM 4-OHT, when the most significant differential growth responses were observed, none of the p53 targets examined displayed a p53-dependent transcriptional response, suggesting that p53 may not be mediating the process through its classical target genes in this context.

The investigators also examined the impact of p53 status on the well-characterized crosstalk between ER and growth factor signaling cascades – a known driver of TAM resistance and estrogen hypersensitivity [2]. Treatment with 4-OHT (1 μM) significantly increased ER-α expression in MUTp53 cells but not WTp53-expressing cells. This increase was associated with a sustained phosphorylation of Akt and p42/44, two signaling kinases downstream of ER and the growth factor receptors, EGF receptor (EGFR) and HER2. Furthermore, the proliferative effects of 4-OHT observed in cells with MUTp53 were abrogated when ER-α was silenced by specific siRNA, or when EGFR/HER2 activity was attenuated using specific inhibitors (such as lapatinib or gefitinib) or by silencing HER2 expression.

Expert commentary

A major challenge in the development of novel approaches to maximize the endocrine response and overcome endocrine resistance is that although estrogen has been implicated as a major etiological factor in breast cancer development and progression, the downstream effectors of its actions remain to be fully characterized. In addition, the complexity of the endocrine response is further impacted by ligand- and DNA-independent activation of ER through crosstalk with cell-surface tyrosine kinase receptors, such as EGFR and HER2, and their downstream signaling cascades [3]. From a clinical perspective, delineating how genes found commonly mutated in breast cancer can influence the response to endocrine therapies is critical in evaluating their predictive value and thus their potential utility in improving patient stratification for more effective treatment.

Mutations in the tumor-suppressor gene, *TP53*, occur in a significant proportion of breast cancers [4] – predominantly in ER-negative tumors – and are an independent predictor of poor prognosis [5,6]. Furthermore, studies by Miller *et al.* using a p53 transcriptional signature identified a subset of aggressive breast tumors that had lost functional p53 signaling, even in the absence of detectable p53 mutations [7], suggesting that p53 dysregulation may exert a greater impact on breast cancer progression than predicted from mutational analyses alone. More recently, a similar approach by Coutant and colleagues utilized distinct p53 signatures derived from ER-positive and ER-negative cancers to demonstrate that p53 dysfunction had greater prognostic and predictive value in ER-positive cancers [8]. The functional links between p53 status and ER signaling have been further highlighted by the demonstration that WTp53 transcriptionally regulates the ER through direct binding to the proximal promoter [9], and can influence both ER-α expression and TAM responsiveness *in vivo* [10].

In the paper under evaluation, Fernandez-Cuesta *et al.* have further explored this functional relationship by determining the impact of p53 status on the cellular response to estradiol and anti-estrogens, and delineating the underlying molecular mechanisms involved [1]. They demonstrated that a loss of functional p53 was associated with TAM resistance and hypersensitivity to the proliferative effects of estradiol, but did not affect the cellular response to fulvestrant. Interestingly, this p53-dependent response could be attenuated by blocking HER2 or EGFR signaling, even in cells that do not overexpress these receptors. These data not only highlight the importance of ligand-independent activation of ER through growth factor receptors as a driver of TAM resistance [2], but also suggest that a loss of functional p53, which is highly correlated with HER2 amplification in breast tumors, may impinge upon and enhance this process, thus providing a molecular basis for the extremely poor prognosis observed in these patients. Furthermore, this may indicate the potential therapeutic efficacy of signal transduction inhibitors in tumors harboring *TP53* mutations, regardless of their HER2/EGFR status.

Five-year view

Endocrine resistance in ER-positive breast cancer remains a significant clinical problem and a major obstacle to the successful treatment of this disease [11]. Delineating the molecular aberrations that drive endocrine resistance is critical to the development of new therapeutic strategies aimed at maximizing the endocrine response in all patients and successfully treating those with refractory disease. Such new knowledge will not only provide us with greater insight into how therapeutic resistance develops, but also has the potential to be rapidly translated into clinical benefit in

two key areas, through developing these molecules as 'biomarkers' of resistance/response. This will enable clinicians to determine at the point of diagnosis which patients are likely to benefit from anti-estrogen therapy, saving those 'nonresponders' from unnecessary treatment and associated significant side effects. The latter can then benefit from early intervention with chemotherapy; and through the development of new drugs – or the better application of existing therapies – that target these molecules and as such have significant potential to lead to the effective treatment of patients with resistant disease.

However, a continuing challenge over the next 5 years will be how to effectively translate the *in vitro* findings of studies, such as that by Fernandez-Cuesta *et al.*, into informative biomarkers and/or therapeutic targets with relevance and utility in the clinical setting. While the research effort over recent years has seen an enormous increase in our understanding of the complexities of breast cancer biology and the drivers of endocrine responsiveness, therapies targeting components of these pathways have often fallen short in the clinic [12]. The continued development of relevant experimental systems that more effectively reflect the heterogeneity of breast cancers and more closely align with clinical material will be crucial for advances in the treatment of endocrine-refractory breast cancer to be realized.

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Key issues

- While endocrine therapies such as the anti-estrogens, tamoxifen and fulvestrant, have had a significant impact on improving survival for breast cancer patients, *de novo* or acquired therapeutic resistance remains a major clinical problem.
- The tumor-suppressor gene, *TP53*, is frequently mutated in breast cancers and is associated with poor prognosis.
- p53 status influences the response of estrogen receptor-positive breast cancer cells to 4-hydroxytamoxifen (4-OHT), but not to fulvestrant.
- Breast cancer cells with mutated or inactivated p53 protein display resistance to the cytotoxic effects of 4-OHT and even become growth-stimulated at low concentrations of this agent.
- This p53-dependent differential response to 4-OHT is mediated via enhanced crosstalk between estrogen receptor and EGF receptor/HER2 signaling pathways.
- In breast cancer tissues, HER2 amplification is significantly associated with *TP53* mutations, and breast cancers with both aberrations have the worst prognosis.
- This study further delineates the predictive value of p53 status in therapeutic response of breast cancers, and may suggest potential novel approaches to the treatment of breast cancers harboring *TP53* mutations.

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