Overexpression of HER-2 as a resistance mechanism to hormonal therapy for breast cancer

M Dowsett
Department of Biochemistry, Royal Marsden Hospital, London SW3 6JJ, UK
(Requests for offprints should be addressed to M Dowsett; Email: mitch@icr.ac.uk)

Abstract
Hormonal therapy leads to improved survival in oestrogen receptor (ER) positive early breast cancer and long-term responses in advanced disease. However, resistance to such therapy is a serious clinical problem. This article considers the data for and against there being a significant role for the oncogene HER-2 in such resistance. Transfection of HER-2 into MCF-7 cells leads to resistance to tamoxifen but data differ in relation to the oestrogen dependence of such cells. A number of retrospective studies have been conducted of HER-2 status in adjuvant trials of tamoxifen. Most of these also suggest a negative role but individually the studies do not have the statistical power to be conclusive. Recent studies in the neoadjuvant context have shown a significant antiproliferative effect of endocrine therapy in HER-2 positive/ER positive tumours but this is much less than in HER-2 negative/ER positive tumours. It is concluded that incomplete hormonal resistance results from co-expression of HER-2 and ER and that this may differ between different hormonal agents.

Introduction
Oestrogens are the most important growth stimulant to breast cancer. About 75% of breast carcinomas express significant levels of oestrogen receptor (ER) (α) and in patients with assessable disease most, but not all, of these respond to some means of abrogating oestrogen signalling. For most patients with early disease, this amounts to the use of tamoxifen (a selective ER modulator; SERM) as adjuvant therapy, whilst oestrogen deprivation with gonadotrophin-releasing hormone agonists (in premenopausal patients) or aromatase inhibitors (postmenopausal patients) are more commonly given for metastatic disease. In general terms, treatment is very well tolerated and responses are of longer duration than to cytotoxic chemotherapy. However, almost all patients with metastatic disease and about 40% of patients on adjuvant hormonal therapy eventually relapse.

The mechanisms for the primary (de novo) and acquired resistance to hormonal therapy are probably numerous. ER negativity at presentation is a major mechanism of de novo resistance with very few patients with such tumours responding (McGuire 1980). Many of these tumours overexpress epidermal growth factor receptor (EGFr) and/or human epidermal growth factor receptor-2 (HER-2), two of the members of the type 1 growth factor receptor family and these appear to provide proliferation/survival signals in the absence of the ER pathway. The expression of these two growth factor receptors is inversely correlated with ER expression (Dowsett et al. 2001) (Table 1). This is more extreme with EGFr, such that very few tumours express both ER and EGFr, but about half of patients that are HER-2 positive are ER positive (about 10% of patients overall). Whether HER-2 overexpression in this ER positive group leads to de novo or acquired hormonal resistance is of substantial contemporary importance: the answer to this

| Table 1 The relationship between ER expression and HER-2 positivity. Tumours were characterised by immunohistochemistry using the DAKOIDS antibody to ER (Saccani-Jotti et al. 1990) and ICR 12 antibody to HER-2 (Styles et al. 1990). ER negative: H-score 0; poor: H-score >0, <20; positive: H-score 20–300 (Dowsett et al. 2001). HER-2 positive: any specific membrane staining. Percentages of the total in respective ER categories are given in parentheses. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **HER-2**       | **Negative**    | **Poor**        | **Positive**    | **Total**       |
| Positive        | 63 (68%)        | 17 (27%)        | 51 (9%)         | 131 (16%)       |
| Negative        | 133 (32%)       | 47 (73%)        | 502 (91%)       | 682 (84%)       |
| **H-score, histopathological score.** | 196              | 64              | 553             | 813             |
question would allow more appropriate delivery of hormonal therapy and would indicate the potential for the combination of endocrine therapy with one of a number of new therapies such as signal transduction inhibitors or HER-2-directed antibody therapy. This article seeks to draw together the available data on this issue and to consider the merits and demerits of this in establishing HER-2 as a significant determinant of endocrine resistance in ER-positive disease.

Experimental studies in model systems

The first reports of the effect of HER-2 transfection into hormone-dependent breast cancer cells were from Benz et al. (1992). Their characterisation of clones of normally hormone sensitive ER-positive MCF-7 cells transfected with HER-2 revealed that those cells with very high gene amplification (45) were tamoxifen resistant but remained oestrogen dependent (i.e. they would not grow in the absence of oestrogen). Similar work by Pietras et al. (1995) confirmed the tamoxifen resistance of HER-2 transfected MCF-7 cells but, in this case, growth \textit{in vitro} was also found to be independent of oestrogen, although this was not examined \textit{in vivo}. Importantly, overexpression of HER-2 or its activation with heregulin led to down-regulation of ER (consistent with the negative clinical correlation referred to above) and ligand-independent increased ER phosphorylation and transcriptional activation, providing a potential mechanism for the tamoxifen resistance. Signal transduction of HER-2 is through the mitogen-activated protein (MAP)-kinase pathway and blockade of this using the MAP-kinase inhibitor U0126 has been found to restore the inhibitory effect of tamoxifen on ER-mediated transcription and cell proliferation in MCF-7 cells transfected with HER-2 (Kurokawa et al. 2000). The use of a monoclonal antibody against HER-2 reversed the tamoxifen resistance in MCF-7 HER-2 transfected cells (Pietras et al. 1995) and in ML-20 breast cancer cells which naturally express moderate levels of HER-2 and ER (Kunisue et al. 2000).

Thus there is compelling \textit{in vitro} evidence that forced expression of HER-2 can lead to the reduced sensitivity of ER positive breast cancer cells to tamoxifen, which may be dependent on ligand-independent phosphorylation of ER. There is some, but much less, evidence that non-transfected overexpression of HER-2 has similar consequences.

Nicholson’s group have found, however, that the development of tamoxifen resistance in MCF-7 cells is associated with increased expression of HER-2 and EGFr and that this provides significant proliferative signalling to the cells (Nicholson et al. 2001).

Clinical studies

There are now several clinical studies which report on the interaction between HER-2 and hormonal resistance but the conclusions from these are disconcertingly varied. This is probably due to a number of factors.

1. The negative correlation between HER-2 and ER (Table 1) which has two consequences:
   a. if ER is not measured or considered as a co-variate, resistance to therapy may be ascribed to HER-2 positivity rather than ER negativity;
   b. only a small group of breast cancer patients are ER positive/HER-2 positive and this drastically reduces the statistical power of most studies.
2. Variable disease settings and therefore different end-points: metastatic, adjuvant, locally advanced, neoadjuvant.
3. A very wide variety of techniques and no standardised cut-off for positivity for HER-2: immunohistochemistry (with a variety of antibodies), fluorescence \textit{in situ} hybridisation, Southern blotting, enzyme-linked immunosorbent assays (ELISAs) for HER-2 fragment in plasma.
4. The recurrent lesion in which tamoxifen relapse is established is usually at a distant site from the primary lesion in which the diagnosis of HER-2 status has been made, such that a different HER-2 status cannot be ruled out.
5. In some cases, the endocrine therapy has been combined with chemotherapy which itself may interact with HER-2.

Our own studies illustrate some of the difficulties involved. We assessed the expression of HER-2 by immunohistochemical means in paraffin-embedded sections from 155 patients whose disease was progressing on tamoxifen (Newby et al. 1997). Fifty-six were known to have initially responded to therapy while 39 had never responded. The other 60 patients relapsed on adjuvant tamoxifen therapy which did not allow initial response to be documented. In 61 cases, pretreatment specimens were also available for direct longitudinal assessment of HER-2. None of the pretreatment samples from 18 patients who responded to treatment expressed HER-2 but four of the 18 non-responders were HER-2 positive ($P = 0.11$). Thus, there was a trend to HER-2, indicating reduced likelihood of response to tamoxifen. Notably, however, at the time of disease progression there was no significant change in HER-2 expression, indicating that such expression infrequently occurs with acquired resistance.

The best clinical scenario to consider a potential interaction between HER-2 and treatment effectiveness in the adjuvant setting is within the context of a randomised trial of tamoxifen versus no treatment as in our retrospective collection of histopathological blocks from the mature Nolvadex Adjuvant Trials Organisation (NATO) and Cancer Research Campaign (CRC) trials. This also had the advantage of having a relatively large number of samples.
(813 tested) and ER status was also available on all of them, allowing ER negative tumours to be excluded from the data analysis. The antibody used had previously been optimised in its immunohistochemical application to be positive only in those tumours with > threefold amplification (Styles et al. 1990). HER-2 was overexpressed in 131 (16.1%) of these and, as a single variable, was associated with significantly reduced benefit from tamoxifen, but only 68 HER-2 positive samples were also ER positive. Thus, although there was no significant improvement in relapse-free survival in this group, the 95% confidence interval extended to a possible 39% reduction in risk of relapse, which is consistent with the benefit seen in the ER positive/HER-2 negative group (M Dowsett, J Houghton, C Iden, J Salter, J Farndon, R A’Hern & M Baum, unpublished observations).

The possible negative effect of HER-2 tamoxifen benefit in the adjuvant setting is of potentially major significance, particularly since one study (Bianco et al. 1998) suggests that tamoxifen may in fact be harmful in these circumstances. However, for there to be confidence in the results produced, the number of events in the HER-2 positive/ER positive group needs to be much greater than has been available from a single trial. This will require the collaborative pooling of data for overview analysis by all groups that have conducted HER-2 analysis in this setting.

There have been three reports of studies of hormonal therapy of metastatic disease in which the extracellular domain (ECD) of HER-2 has been measured by ELISA in patients’ plasma and, in each case, the patients with higher HER-2 ECD had a poorer outcome. Leitzel et al. (1995) found 19.3% of 300 metastatic breast cancer patients (treated with either megestrol acetate or fadrozole) to have elevated HER-2 ECD. The response rate to the endocrine therapy in the 242 patients with low levels was 40.9% compared with only 20.7% in the 58 patients with high levels (P = 0.004). Yamauchi et al. (1997) reported a greater differential response rate in their smaller trial of the SERM droloxifene: only three of 32 patients (9%) with elevated ECD levels responded to droloxifene compared with 35 of 62 (56%) with non-elevated levels (P = 0.00001). Most recently, Lipton et al. (2000) reported that 168 of 566 patients in two second-line hormone therapy trials had elevated HER-2 ECD levels and that these had a 24% response rate compared with 44% in the 398 patients with lower levels. These trials did not include known ER negative patients but ER unknown patients were included. While these data strongly support a poor response to endocrine therapy of patients with high HER-2 ECD this may be, at least in part, because HER-2 positive tumours proliferate more rapidly: thus an equivalent biological response may not translate to a clinical response similar to that seen in HER-2 negative tumours.

More recently, studies have been conducted of endocrine resistance in the neoadjuvant setting which has the substantial benefit that, unlike in the adjuvant therapy, clinical outcome data are based on response or lack of it. This means that fewer patients are required for significant results and that HER-2 (and ER) status can be measured in the same lesion in which response is assessed. In our own studies, the antiproliferative response to a set of different hormonal therapies was markedly and significantly lower in ER positive/HER-2 positive patients than in ER positive/HER-2 negative patients (Dowsett et al. 2000). Nonetheless, there was a significant reduction in proliferation in the former group but, when associated with the much higher pretreatment proliferation levels of the HER-2 positive group, this was considered likely to translate to clinical response in only a minority of patients. There was no indication that there was an acceleration of proliferation with anti-oestrogen therapy. This clinical study thus suggested that HER-2 positivity leads to a quantitative but not absolute resistance to endocrine therapy. This implies that endocrine treatment should not necessarily be withheld from HER-2 positive patients but that combination with HER-2-directed therapy might give additive benefit.

A randomised neoadjuvant multicentre trial of the aromatase inhibitor letrozole versus tamoxifen in over 300 ER positive primary breast cancer patients not suitable for breast conserving surgery is of particular interest (Ellis et al. 2000). Those patients who had tumours that were EGFr and/or HER-2 positive as well as being ER and/or progesterone receptor positive had a significantly greater response to letrozole than to tamoxifen (88% vs 21%). Additionally, response to letrozole was significantly higher in the patients with EGFr and/or HER-2 positive tumours than in those with tumours that were negative for both receptors. These are important data which, if confirmed, could significantly alter the recommended treatment for patients with ER positive EGFr or HER-2 positive tumours. There is a hypothetical molecular rationale for such a differential effect: tamoxifen binds to ER to lead to a conformational change which determines the association or not of ER with one of a large series of co-activators or co-repressors which determine the resultant agonist or antagonist effect on the transcription of particular genes. Deprivation of oestrogen ligand leads to different conformational change and a pure antagonist effect (Connor et al. 2001). It is plausible that the consequences of these differences may be substantially affected by the presence of alternative down-stream signalling pathways from EGFr/HER-2, e.g. ER phosphorylation. Differential effects of oestradiol deprivation and tamoxifen treatment on ER negative-mediated transcription have been reported in HER-2 amplification in MCF-7 cells in support of this hypothesis (Kurokawa et al. 2000) (Fig. 1).

Conclusion

Model systems indicate that forced overexpression of HER-2 can lead to tamoxifen resistance in ER positive breast cancer
cells. The clinical data are less clear but overall point to incomplete resistance resulting from co-expression of HER-2 and ER. Recent data suggest that the effect of HER-2 may differ according to the nature of the endocrine treatment. The rational application of signal transduction inhibitors to alleviate resistance depends on the better characterisation of this interaction with these individual agents.

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References


hormone therapy in metastatic breast cancer. *Proceedings of the American Society of Clinical Oncology* **18** 71A.


