Growth factor signalling in clinical breast cancer and its impact on response to conventional therapies: the Edinburgh experience

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Abstract

Neoadjuvant endocrine treatment in which therapy is given while the primary tumour is still in the breast provides a highly useful model system by which to identify mechanisms associated with de novo resistance and signs of early acquired resistance. Most importantly, the model is clinically relevant. It has been confirmed that the absence of tumour oestrogen receptors confers resistance to endocrine therapy. Early changes in tumour cell proliferation following neoadjuvant treatment with the third-generation aromatase inhibitor, letrozole, do not predict accurately for subsequent clinical response. Additionally, changes in proliferation seen at later times can be the consequence of response and may be associated with early resistance. High expression of c-erbB2 does not reduce tumour responses to neoadjuvant treatment with aromatase inhibitors, but is associated with high tumour proliferation before and during treatment. It remains to be determined whether these characteristics confer subsequent resistance to treatment and early relapse in the adjuvant setting.

Introduction

The Edinburgh Breast Unit has a major interest in identifying predictive indices of early response and resistance to endocrine therapy and their underlying mechanism of action. To investigate these in relevant clinical material we have exploited the use of therapy given in the neoadjuvant setting (Forrest et al. 1986, Miller et al. 1999). The basis of neoadjuvant systemic treatment is that the conventional sequence of breast surgery followed by systemic therapy is switched, such that systemic therapy is given with the primary tumour still residing in the breast. The approach may yield clinical benefits by (i) down-staging large tumours so that ultimately more conservative surgery can be given and (ii) providing the opportunity for subsequent adjuvant treatment to be based on the knowledge of tumour sensitivity to a trial therapy (patients with non-responding tumours might also benefit by avoiding the unnecessary side-effects of extended adjuvant treatment with an ineffective agent). However, neoadjuvant therapy also has particular attractions for research in that accessibility of the primary tumour means that (i) tumour size can be measured precisely to provide accurate response data, (ii) pre-treatment biopsies taken for diagnosis may be analysed for predictive markers of response/resistance and related to volume changes of the same tumour during treatment and (iii) sequential samples can be taken from the tumour before, during and after treatment to monitor pathological and molecular changes associated with therapy.

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Response to endocrine therapy

Initially, 89 patients were recruited for treatment irrespective of oestrogen receptor (ER) status. However, only 1 of 27 ER-poor tumours (defined as <20 fmol/mg cytosol protein) responded to various endocrine therapies (which included oophorectomy and LHRH agonists in premenopausal women and aminoglutethimide, 4-hydroxyandrostenedione and tamoxifen in postmenopausal patients). In contrast, 35 of 62 ER-rich tumours (>20 fmol/mg cytosol protein) regressed within 3 months of treatment. Because of this, the decision was taken to restrict subsequent endocrine therapy to patients with ER-rich tumours.

Because the presence of ER does not guarantee response, optimal management with endocrine therapy requires the identification of further markers by which to subdivide ER-rich tumours into responders and non-responders. Our experience examining the utility of progesterone receptor (PgR) status on response to neoadjuvant treatment with either tamoxifen or an aromatase inhibitor is shown in Table 1. Thus, whereas the presence of PgRs in ER-positive tumours increases the likelihood of response, the PgR-negative groups also had substantial response rates.

Other markers may only be predictive effects for certain types of endocrine therapy. Thus in the P 024 study (Ellis et al. 2001), in which patients were randomized to receive either tamoxifen or letrozole, high expression of epidermal growth factor receptor and/or c-erbB2 was associated with a decreased likelihood of the response to tamoxifen but an increase of the response rate to letrozole. Results from Edinburgh in which patients were treated with a third-generation inhibitor (anastrozole, letrozole or exemestane) are summarized in Table 2. Overexpression of c-erbB2 did not appear to be associated with resistance to any of the aromatase inhibitors. Overall, response rates were similar irrespective of c-erbB2 expression.

Changes induced by treatment

The proliferation marker, Ki-S1 (Miller et al. 1999), was measured by immunohistochemistry in tumours from elderly patients with ER-rich tumours before and after 3 months of treatment with tamoxifen. Changes in the proportion of staining tumour cells were categorized as increasing, decreasing or not having changed, as described previously (Keen et al. 1997), and related to clinical response as shown in Table 3. The majority of responding tumours showed a decrease in Ki-S1 with treatment whereas most non-responding cancers displayed either no change or an increase in staining; this difference was statistically significant between the groups (P = 0.0003, by Fisher’s exact test). However, there were smaller groups of tumours showing changes in proliferation inconsistent with their clinical response; responding tumours that displayed no change or an increase in proliferation and non-responding tumours that had decreased proliferation with treatment. All patients received tamoxifen in the adjuvant setting and now have at least 5 years of follow up. Incidence of recurrent disease was higher in the 14 non-responding tumours (50%) than the 37 responsive tumours (22%). Interestingly, however, the small group of eight patients who, despite responding to treatment, failed to show a decrease in Ki-S1, had a recurrence rate identical to the non-responding tumours. It may be that high levels of Ki-S1 following successful therapy represents the outgrowth of tamoxifen-resistant, highly proliferative cellular clones and may be a marker of a resistant and

### Table 1 Response to aromatase inhibitors: PgRs

<table>
<thead>
<tr>
<th>PgR status</th>
<th>Tamoxifen</th>
<th>Aromatase inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>81% (29/36)</td>
<td>88% (46/52)</td>
</tr>
<tr>
<td>Negative</td>
<td>43% (6/14)</td>
<td>57% (4/7)</td>
</tr>
</tbody>
</table>

### Table 2 The relationship between clinical response to aromatase inhibitors and c-erbB2 expression as assessed by immunohistochemistry

<table>
<thead>
<tr>
<th>c-erbB2 status</th>
<th>Letrozole</th>
<th>Anastrozole</th>
<th>Exemestane</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>N-R</td>
<td>R</td>
<td>N-R</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>54</td>
<td>14</td>
<td>13</td>
<td>4</td>
</tr>
</tbody>
</table>

P = N.S.

R, responsive; N-R, non-responsive; N.S., not significant.
aggressive phenotype. If this is the case, changes observed after 3 months of treatment may be the consequence of response rather than the cause of it. Certainly, such changes cannot be used as predictors of response that are usually clinically apparent at 3 months.

In order to identify predictive markers of response, it is necessary to examine the tumour at an earlier time point. To do this we have adopted the experimental protocol illustrated in Fig. 1. In this, additional core biopsies are taken at 10–14 days for research purposes. Compared with pre-treatment samples, these biopsies show a marked decrease in expression of PgRs following treatment with the third-generation aromatase inhibitor letrozole in about 80% of cases, this being a total disappearance in 40%. This effect is evidence of the rapid and profound anti-oestrogenic properties of novel aromatase inhibitors.

It was also of interest to look at patterns of proliferation in this model. To date 63 patients have been offered neoadjuvant treatment with letrozole and tumour proliferation assessed by immunohistochemical staining with the Ki67 antibody against MIB1 antigen in pre-treatment, 10–14-day and 3-month treatment biopsies. These results are presented in Fig. 2. Of the 63 cases, 52 (82.5%) showed a decrease in proliferation (> 40% reduction in Ki67 staining) at 10–14 days. Of the 11 cases failing to show this decrease, seven were tumours that were classified as having a clinical response at 3 months and four were non-responders. Early changes in proliferation are therefore not accurate predictors of subsequent response. Comparison of measurements at 3 months with those before therapy indicated that 54 tumours were classified as having decreased proliferation with this period of treatment; this included six cases that had not shown a reduction at 10–14 days. Of the 10 tumours not showing decreased proliferation at 3 months, five had been unchanged or increased at 10–14 days (these comprised four clinical responders and one non-responder) whereas five had shown a decrease at 10–14 days but a rise between 10–14 days and 3 months meant that there was no difference between pre-treatment values (the cases comprised three clinical responders and two non-responders). It can be seen that changes in proliferation at 3 months were not related to clinical response. Follow-up data on these patients are not available at this time, but it is

<table>
<thead>
<tr>
<th>Proliferation phenotype</th>
<th>Total</th>
<th>Decrease</th>
<th>No change/increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24% Responders</td>
<td>17% (5/29)</td>
<td>50% (4/8)</td>
<td></td>
</tr>
<tr>
<td>50% Non-responders</td>
<td>67% (2/3)</td>
<td>45% (5/11)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Recurrence rates at 5 years in patients subdivided according to clinical response to neoadjuvant tamoxifen and proliferation phenotype as assessed by changes in Ki-S1 staining.

Figure 1 Research protocol for neoadjuvant therapy.
of interest to determine and compare the patterns of molecular changes in those tumours that showed decreased proliferation at 3 months (proliferation responders) with those having no evidence of decreased proliferation at either 10–14 days or 3 months (proliferation de novo-resistant) and those that decreased at 10–14 days but not 3 months (proliferation acquired-resistant). This is considered below.

The c-erbB family of growth factor receptors

A similar group of 56 patients (including most of those studied for tumour proliferation) were investigated for changes in tumour genetic profiles as assessed by microarray analysis. A preliminary account of this profiling has been reported elsewhere (Miller & Larionov 2005). However, it was of interest to examine specifically the expression of the c-erbB receptor family in this series and the relationship with clinical response and changes in proliferation following neoadjuvant treatment with letrozole.

Although c-erbB4 was present on the array, expression was very low in most tumours and was called as being absent or marginal. In contrast, c-erbB3 was expressed in all cases. In 14 tumours, levels of c-erbB3 before treatment were at least 5-fold higher than the minimum detectable level. Interestingly, this high expression was more associated with non-responding tumours (six of 16 (37.5%) non-responders and eight of 40 (20%) responders) but the trend did not reach statistical significance. Two tumours displayed a more than 2-fold increase in treatment at 3 months; both were classified as clinical responders but one had a proliferation phenotype of acquired resistance.

With regard to c-erbB1, expression was low or absent and only two tumours were positive at pre-treatment. However, expression was induced by treatment in five cases: all were clinical responders that displayed reduced proliferation at both 10–14 days and 3 months. There is therefore no evidence to suggest that increased expression with treatment is associated with clinical resistance to letrozole.

c-erbB2 was expressed in most tumours but level of expression was very variable. Four cases had high expression in the pre-treatment biopsy. These comprised two responders and two non-responders but three had a de novo resistance proliferation phenotype; i.e. treatment did not substantially reduce proliferation either at 10–14 days or 3 months (see Fig. 3). Three cases displayed an increase in expression of more than 2-fold after 3 months of treatment; these represented a clinical responder with decreased proliferation on treatment, a clinical non-responder with no change in proliferation on treatment and a clinically unassessable case with no reduction in proliferation. Whereas c-erbB2 expression did not predict for clinical response, there is the impression that the high expression is associated with high tumour-cell proliferation even in the face of treatment causing tumour shrinkage. Only data from clinical follow up will reveal whether this is associated with early resistance to treatment and poor prognosis.

Concluding remarks

Neoadjuvant treatment provides a useful clinical model and the opportunity to obtain primary tumour material by which to explore molecular mechanisms associated with de novo resistance and early acquired...
resistance. The model has already demonstrated that the absence of tumour ER confers endocrine resistance. In this review there are also suggestions that high expression of c-erbB2 is associated with high cellular proliferation even after effective oestrogen deprivation. Whether this translates eventually into endocrine resistance and a poor outcome remains to be determined. The present studies are not definitive and require larger groups of patients. It should also be noted that whereas the particular protocol involving neoadjuvant therapy for 3 months can provide evidence of de novo resistance and early forms of acquired resistance, it is unlikely to be useful in identifying processes that occur in the longer term.

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**References**


