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Tamoxifen Resistance in Breast Cancer

Elucidating Mechanisms

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Abstract

Tamoxifen has been used for the systemic treatment of patients with breast cancer for nearly three decades. Treatment success is primarily dependent on the presence of the estrogen receptor (ER) in the breast carcinoma. While about half of patients with advanced ER-positive disease immediately fail to respond to tamoxifen, in the responding patients the disease ultimately progresses to a resistant phenotype.

The possible causes for intrinsic and acquired resistance have been attributed to the pharmacology of tamoxifen, alterations in the structure and function of the ER, the interactions with the tumour environment and genetic alterations in the tumour cells. So far no prominent mechanism leading to resistance has been identified.

The recent results of a functional screen for breast cancer antiestrogen resis-

tance (*BCAR*) genes responsible for development of tamoxifen resistance in human breast cancer cells are reviewed. Individual *BCAR* genes can transform estrogen-dependent breast cancer cells into estrogen-independent and tamoxifen-resistant cells *in vitro*. Furthermore, high levels of *BCAR*1/p130Cas protein in ER-positive primary breast tumours are associated with intrinsic resistance to tamoxifen treatment. These results indicate a prominent role for alternative growth control pathways independent of ER signalling in intrinsic tamoxifen resistance of ER-positive breast carcinomas.

Deciphering the differentiation characteristics of normal and malignant breast epithelial cells with respect to proliferation control and regulation of cell death (apoptosis) is essential for understanding therapy response and development of resistance of breast carcinoma.

1. Breast Cancer Development

Breast carcinoma originates from the breast epithelium as a sex steroid hormone-dependent process. Endocrine and reproductive factors have been shown to affect the occurrence of this malignancy[1-4] and women with reduced estrogen production exhibit strongly decreased frequencies of breast cancer. Furthermore, the incidence of sex steroid hormone receptors is higher in the early stages of breast cancer than in more advanced stages of the disease.[5] The action of estrogen is transmitted through the estrogen receptor-α (ERα), which acts as a ligand-activated transcription regulator in concert with many co-regulators.[6-10] In addition, a second estrogen receptor (ERβ) may contribute to gene regulation by estrogen in breast epithelial tissue,[11,12] but its role in breast cancer needs further clarification.[13-19] Apart from deregulation of cell growth, other requirements – such as evasion of apoptosis – have to be met to establish malignant cancer.[20]

2. Endocrine Therapy of Breast Cancer

Estrogen also plays an important role in human breast cancer expansion and progression. More than a century ago it was noted that removal of the ovaries, i.e. reducing endogenous estrogen levels, could induce remissions in patients with advanced breast cancer.[21] Furthermore, over two-thirds of breast carcinomas are positive for ER,[22,23] and could benefit from manipulation of estrogen levels and antiestrogenic action. This has ultimately resulted in the development of the nonsteroidal antiestrogen tamoxifen for the treatment of advanced breast cancer.[24]

The active metabolite of tamoxifen, 4-hydroxytamoxifen, was shown to compete for binding of natural estrogen to the ERα with high affinity.[25] Antagonist binding to ERα elicits a different 3-dimensional structure in the receptor, which renders it unable to enhance specific gene expression.[26] Thus, antiestrogens interrupt the estrogen-induced signals, which can result in inhibition of cell proliferation, tumour growth arrest and induction of apoptosis.[27,28] In addition to its use in advanced breast cancer, tamoxifen is widely applied for adjuvant treatment of breast cancer and significantly reduces disease recurrence in patients with ERα-positive primary tumours.[29] Tamoxifen is currently also prescribed for the prevention of breast cancer in high risk patients.[30-34] Apart from its antiestrogenic effect, agonist activity of tamoxifen has been observed in breast cancer (flare) and other tissues.[35-38]

Various antiestrogens and other selective estrogen receptor modifiers are currently available or under development for specific modulation of estrogen responses to improve the therapeutic effect and to modulate adverse effects on, for example, endometrium and bone.[39,40] Furthermore, combination endocrine treatments have been evaluated[41,42] and are being studied in clinical trials for their potential to prolong tumour response and survival.

In advanced breast cancer, response to tamoxifen

is mainly dependent on the ER status of the tumour. ERα-negative tumours are unlikely to respond to tamoxifen treatment, whereas over half of the ERα-positive tumours exhibit some sort of response.[43] The therapeutic response to endocrine treatment in advanced disease is generally assessed according to the criteria of the International Union Against Cancer (UICC).[44] A minor proportion of patients exhibit complete remission. The majority of patients show either partial remission or stable disease for more than 6 months and both groups of patients have similar survival characteristics.[45-47] The remaining patients with ERα-positive primary tumours experience short term stable disease (less than 6 months), or show immediate disease progression and are considered intrinsically resistant to tamoxifen treatment. Patients who are initially responsive to tamoxifen ultimately exhibit progression of disease, which may occur at the first metastatic site or at another site, and thus present with acquired tamoxifen resistance.

Predictive factors for response to tamoxifen therapy are still rather scarce.[48] Expression of ERα in combination with expression of progesterone receptor (PR) and pS2 are strong positive indicators of response.[47] Furthermore, expression of urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1,[49] HER2/neu,[50,51] epidermal growth factor receptor (EGFR),[52,53] ERβ,[15,16] TP53 overexpression or mutation,[54,55] and high expression of thymidine kinase[56] were found to be associated with poor response to tamoxifen.

In general, development of acquired tamoxifen resistance is not accompanied by loss of ER expression[57-59] or loss of ER binding to DNA.[60] Patients developing acquired tamoxifen resistance still have a fair chance of responding to second- and third-line endocrine manipulation (including pure antiestrogens, progestogens, aromatase inhibitors, antiprogestogens and androgens), while patients who are intrinsically resistant are unlikely to respond.[61,62] The mechanisms underlying intrinsic and acquired tamoxifen resistance of ERα-positive disease are not yet understood and need to be elucidated to allow for development of new classes of agents that lack cross-resistance to standard therapies.[63]

3. Mechanisms of Resistance to Tamoxifen

Three possible mechanisms relating to the frequent development of resistance to tamoxifen treatment have been studied intensely in the past two decades. These are the pharmacology of tamoxifen, alteration in the structure and function of the ER, and the role of the tumour environment. Many reviews have discussed these options[24,64-72] and the conclusions are briefly summarised here.

3.1 Pharmacology of Tamoxifen

Tamoxifen is metabolised in the body into its active form (4-hydroxy-tamoxifen) which is able to compete with the natural estrogen for binding to the ER.[73,74] Alternative metabolising pathways could result in the production of agonistic compounds, which would stimulate tumour growth.[75] Growth stimulation of the tumour by tamoxifen is suggested because of the occurrence of tumour responses in some patients after withdrawal of tamoxifen. Furthermore, oophorectomy is effective only when tamoxifen treatment of the tumour is discontinued.[76] Fixed-ring structure variants of tamoxifen gave similar growth characteristics in tamoxifen-stimulated human breast tumour models,[77,78] indicating that the metabolism of tamoxifen is not the major determinant for drug-induced tumour growth. Neither does the bioavailability of tamoxifen for the tumour cells appear to determine the development of acquired resistance.[79,80] Moreover, a pure antiestrogen fulvestrant (ICI-182780) produces prolonged duration of response, but ultimately resistance will develop.[81,82]

3.2 Structure and Function of the Estrogen Receptor

The prominent role of the ERα in growth regulation, its putative oncogenic potential[83] and the identification of ERα variants have initiated a ma-

jor worldwide investigation into the involvement of ERα in tamoxifen resistance of breast cancer. It is clear from all published studies that mutations of ERα are quite rare.[84-86] In contrast, splice variants of ERα mRNA are found frequently in various cell types expressing the wild-type messenger,[87-92] and variants of the ERβ mRNA have also been identified.[93,94] For ERα, no correlation between the occurrence of specific variants and tamoxifen resistance has been established.[91] However, in individual patients the involvement of a dominantly active ERα variant in therapy failure can not be excluded.

3.3 Paracrine Interactions

Since the tumour cells are embedded within tissue and depend on interaction with the environment,[20] this interaction may affect the treatment outcome. Signals required for tissue remodelling, neo-angiogenesis and invasion are exchanged between the tumour cells and the surrounding tissue. Tamoxifen may affect cells lacking ERα in as yet undefined ways and elicit production of growth factors capable of supporting tumour cell proliferation.[95-100] There is no clear evidence that the tumour cell environment causes tamoxifen resistance. However, the observation that high levels of uPA and PAI-1, which are primarily expressed by the stromal cells, are associated with poor response to tamoxifen treatment[49] emphasises the importance of the tumour environment and our ignorance about the ongoing interactions.

3.4 Genetic Mechanisms

Genetic and epigenetic alterations are important steps in the development of malignancies and may contribute to disease progression during treatment. Similarly, genetic alterations may play a role in development of tamoxifen resistance.[69,101] Searches for genes exhibiting altered expression in breast tumour cells have been performed using differential display, SAGE (serial analysis of gene expression) and array techniques, and have identified several marker genes.[71,102-106] Transfection experiments of putative candidate genes (oncogenes and growth regulatory genes) into estrogen-dependent breast cancer cells have been carried out for many years and have identified a number of genes capable of supporting estrogen-independent or tamoxifen-resistant cell growth.

These genes include activated Ha-*RAS*,[107] insulin-like growth factor II,[108] *EGFR*,[109] *HER2/NEU*,[110] *Heregulin*,[111] *FGF*-1,[112] *FGF*-4,[113] *VEGF*,[114] *TGF*β1,[115] *Cyclin D1*,[116] activated *RAF1*,[117] *IGF-1R* and *IRS1*,[118,119] *CYR61*[120] and activated *AKT*.[121] Furthermore, random deregulation of gene expression by 5-azacytidine treatment also transformed the hormone-dependent phenotype of breast cancer cells.[122] Despite these compelling data on *in vitro* hormone-independent growth control of these dominantly acting genes, evidence for direct involvement in clinical breast cancer progression is still limited. Expression of the EGFR, which is inversely related to ERα, defines a subgroup of essentially ER-negative breast tumours unlikely to respond to tamoxifen.[52] HER2/NEU amplification and/or overexpression is found in approximately 25% of breast carcinomas and predicts failure of tamoxifen therapy.[51,123] Alternative strategies to identify genes responsible for breast cancer tamoxifen resistance by transfer of resistant phenotype using cell fusion[124] and random transfection of cDNA libraries[125] have been applied, but with limited success.

4. Functional Screen for Breast Cancer Antiestrogen Resistance (*BCAR*) Genes

Although each of the mechanisms discussed in section 3 may be involved in tamoxifen resistance in an individual patient, no explanation has been provided for the majority of patients with breast cancer.

In order to find novel genetic leads for the dominant mechanisms in development of antiestrogen resistance in human breast cancer, we have used the human breast cancer cell line ZR-75-1 as a model for estrogen-dependent breast cancer. This cell line is completely dependent on estrogen for cell proliferation. Spontaneous progression of these cells to antiestrogen resistance is extremely

low ($<10^{-8}$) during 4 weeks of selection with antiestrogen.[126] In contrast, alteration of gene expression in this cell line can induce antiestrogen-resistant variants. Introduction of the *EGFR* gene in ZR-75-1 cells conferred estrogen independence and allowed for rapid progression to antiestrogen resistance.[109] In addition, random deregulation of gene expression in ZR-75-1 cells with 5-azacytidine also produced efficient transformation into antiestrogen resistance.[122]

In a random search for (unknown) genes capable of inducing antiestrogen resistance, insertion mutagenesis with defective retrovirus has been applied. Retroviruses integrate into the host cell genome as part of their life cycle.[127] This integration event causes local disruption of the genome structure. Promoter and enhancer elements within the viral long terminal repeats may induce expression of nearby genes located both upstream and downstream of the virus.[128,129] Culture with an antiestrogen may then select for infected cells that have acquired the capacity to grow under these conditions as a result of the integration event near the responsible gene. An important feature of this procedure is the presence of the virus in close proximity to the responsible gene allowing for its identification.

Over 800 million ZR-75-1 cells have been infected with a defective murine retrovirus and subjected to culture with 1 μmol/L of 4-hydroxy tamoxifen for 4 to 5 weeks. Developing surface colonies consisting of proliferating cells were picked and expanded.[126] About 80 cell lines were generated exhibiting resistance to 4-hydroxy tamoxifen.

Two procedures (screening for common integration sites and transfer of loci by cell fusion) have been applied to ascertain the linkage of a particular integration event with the biological phenotype of antiestrogen resistance.

The first procedure establishes the occurrence of viral integration events in the same small genomic region in independently arisen cell lines (common integration site), which are unlikely to occur by chance. In such common integration sites, the position of the virus in the cellular genome is linked to the selected biological phenotype and thus should be very close to the relevant gene.[126,128,129]

The alternative approach was offered by the presence of a selectable marker (neomycin resistance) on the viral genome. Large fragments of genomic DNA from lethally irradiated, antiestrogen-resistant cells were transferred to the parental cells by cell fusion.[130] Selection for G418 resistance (a neomycin analogue) allowed for the rescue of somatic cell hybrids that had retained a DNA fragment containing a copy of the retrovirus. Since only part of the genomic DNA of the donor cells was retrieved in the somatic cell hybrid, the role of a particular integration event could be determined. The two integration loci present in a donor cell line could be separated among different cell hybrids and individually tested for their role in antiestrogen resistance.[130]

So far we have identified three breast cancer antiestrogen resistance (*BCAR*) loci, which are the cause of resistance in 15 cell lines of the panel and are distinct from the hereditary breast cancer genes (*BRCA1* and *BRCA2*).[131,132]

4.1 *BCAR1*

The *BCAR1* locus was identified as a common integration site in 4 independent cell lines.[126] The *BCAR1* locus was mapped to chromosome 16q22-23. Scanning of the *BCAR1* region for genes identified a transcript of 3.2kb highly expressed in the cell lines with a viral integration and virtually undetectable in the parental cells. Transfectants showing expression of this gene displayed resistance to 4-hydroxy tamoxifen and the pure antiestrogen fulvestrant, thus demonstrating that this cDNA represents the *BCAR1* gene.[133]

Sequence analysis identified this gene as the human homologue of the rat Crk-associated substrate protein ($p130^{CAS}$), a major phosphorylation target in v-Src– and v-Crk–transformed rat cells.[134] $p130^{CAS}$ exhibits features of an adaptor protein [including a Src Homology 3 (SH3) domain] involved in intracellular signalling during cell transformation, integrin activation, cell migration and inva-

sion, and many other processes.[134-136] Although BCAR1 protein (referred to below as BCAR1/p130Cas) is capable of bypassing the ER dependence of ZR-75-1 cells, its precise role in antiestrogen-resistant cell proliferation of human breast cancer cells is currently under investigation.

4.2 *BCAR2*

The second locus implicated in antiestrogen resistance was identified following cell fusion-mediated gene transfer.[130] Polymerase chain reaction (PCR) analysis on DNA from a radiation hybrid cell panel and *in situ* hybridisation map the *BCAR2* locus on the human chromosome 6p12.1-21.1. The search for the candidate gene in this *BCAR2* locus has identified up-regulated transcripts of 5 and 8kb, which represent alternatively polyadenylated transcripts. Constructs containing the complete coding region are currently transfected into ZR-75-1 cells to confirm the role of the BCAR2 protein in antiestrogen-resistant cell proliferation (Veldscholte et al., unpublished results).

4.3 *BCAR3*

The third *BCAR* locus was identified by screening for common integration sites on southern blots.[137] PCR analysis and *in situ* hybridisation have shown that this integration locus is located on chromosome 1p21. Sequence information maps this gene at 1p21.2-22.2. This *BCAR3* locus was subsequently shown to cause antiestrogen resistance following cell fusion-mediated gene transfer. A candidate gene (3.4kb mRNA) with increased expression levels in the cell lines with an integration in this locus was isolated and transfected into the parental cell line ZR-75-1. Transfectants exhibit resistance to 4-hydroxy tamoxifen and the pure antiestrogen fulvestrant, confirming the identity of this gene as the *BCAR3* gene. Transfection of the *BCAR3* gene into another human breast cancer cell line (MCF7) also conferred resistance to pure antiestrogen (fulvestrant) to these cells.

Sequence analysis of the *BCAR3* cDNA revealed a novel gene product containing an Src homology 2 (SH2) domain and partial homology to the cell division cycle protein 48 of bacteria, yeast and mammalian species, and a putative guanosine diphosphate (GDP)-exchange factor (GEF) domain. Northern analysis revealed that in a panel of 24 cell lines derived from malignant human breast, ovarian and endometrium tissue, and 2 cell lines from nonmalignant breast tissue, *BCAR3* mRNA expression was inversely related to ER expression ($p = 0.025$).[137]

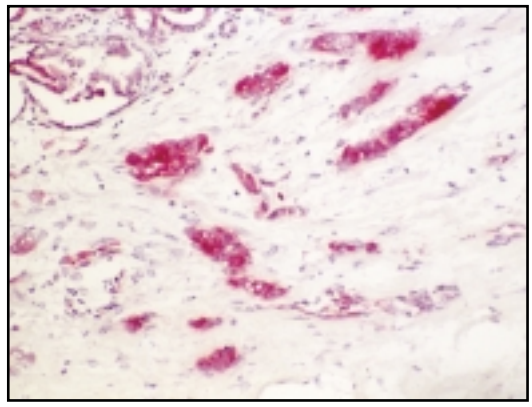

Fig. 1. BCAR1/p130Cas protein in breast cancer. A section of a breast tumour specimen (intraductal carcinoma) was stained for BCAR1/p130Cas (red) and the nuclei were counterstained with Mayer haematoxylin (blue). BCAR1/p130Cas staining is detectable in the malignant and nonmalignant epithelial cells, and in endothelial cells of blood vessels. No staining is seen in the stromal compartment (courtesy of M. Timmermans).

4.4 *BCAR1* and Prognosis of Breast Cancer

Expression of the BCAR1/p130Cas protein has been analysed by western blotting in a large series ($n = 775$) of patients with primary breast tumours and was found to vary considerably. These observations are in agreement with the variable levels of BCAR1/p130Cas protein in ER-positive and ER-negative malignant and nonmalignant breast epithelial cells detected with immunohistochemical analysis (fig. 1). Expression of BCAR1/p130Cas was also found in blood vessels, but no significant immuno-staining was observed in the stromal cell layer between the epithelial cells. The western blotting expression data of the breast tumours have

been combined with the clinical data of the patients.[138]

Both in univariate and in multivariate analysis (including age/menopausal status, nodal status, tumour size and steroid-hormone receptor status), a high expression level of BCAR1/p130Cas in 8% of the primary breast tumours was associated with an increased rate of relapse (relative hazard rate = 1.62; multivariate p value = 0.03). In addition, a high expression level of BCAR1/p130Cas was associated with a poor response to first-line tamoxifen therapy in 268 patients with recurrent disease, also when corrected for age/menopausal status, disease-free interval, site of relapse, and steroid-hormone receptor status (odds ratio = 0.38; multivariate p value = 0.044). Patients with tumours with high BCAR1/p130Cas levels (nearly all ER-positive) showed reduced response frequencies (33%) compared with patients containing tumours without (53%) or with low/intermediate levels (53%) of BCAR1/p130Cas (fig. 2). Thus high expression of BCAR1/p130Cas in the primary tumours is a marker (independent of other clinical parameters) of poor clinical behaviour of the disease.[138]

Additional studies on a limited number of samples so far have not revealed significant differences in BCAR1/p130Cas expression in acquired resistant breast carcinomas compared with untreated tumours.[139]

5. Clues for Mechanisms of Antiestrogen Resistance in Breast Cancer

On the basis of the observations in our cell line model and patient specimens, we hypothesise that the response of breast cancer to antiestrogen treatment is determined by the differentiation properties retained by the tumour cells. Firstly, sex steroid hormone receptor-negative tumours are unlikely to respond to antiestrogen, since their growth is regulated completely independently of ERα function. We have presented evidence that individual *BCAR* genes can control cell proliferation *in vitro* independently of estrogen-mediated signalling.[126,130,133,137] Furthermore, highly variable levels of BCAR1/p130Cas protein were found in malignant breast tumours and determined the type of response to tamoxifen treatment.[138] These variable levels of BCAR1/p130Cas were also found in nonmalignant breast epithelial cells and may correspond to the growth regulatory programme of the particular cell. In the following sections (5.1 to 5.3), we discuss some aspects of breast epithelial cell differentiation as they affect growth control, cell death and tamoxifen resistance.

5.1 Proliferation Control and Breast Epithelial Cell Differentiation

Contrary to our detailed knowledge of haematopoietic differentiation, little information is available for the differentiation programme of breast epithelium. This is mainly attributable to the ab-

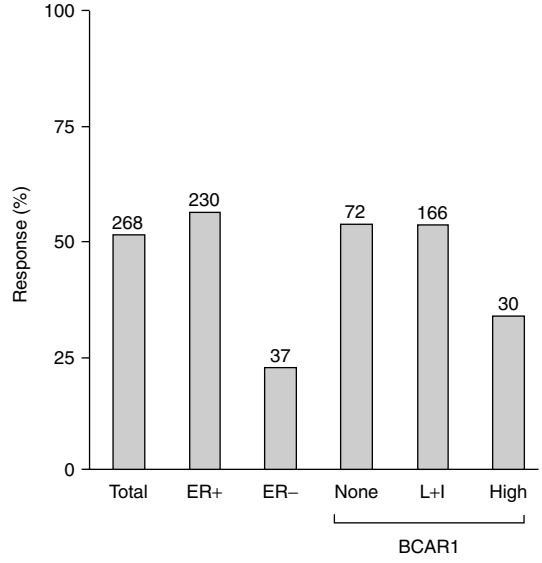

Fig. 2. Response (%) of patients with advanced breast cancer to tamoxifen treatment. The response frequencies of patients with advanced disease are depicted for the various categories of primary tumours. Numbers above the columns denote the number of patients. BCAR1/p130Cas protein levels of the primary tumours are categorised as none, low plus intermediate (L+I) and high. Note that the subgroup of high BCAR1/p130Cas consists primarily of patients with estrogen receptor-positive (ER+) primary tumours (29 of 30). [Adapted from data published by Van der Flier et al.[138]]

sence of large quantities of pure, uncultured malignant cells and convenient *in vitro* assays for research, a requirement which was fulfilled for various haematopoietic malignancies.[140]

So far, major distinctions in breast epithelial cell types have been based on cell morphology and cell localisation within the duct, expression of cell skeletal proteins, presence of the EGFR or the ERα, and on studies in mouse transgenics.[4,140-144] Parts of these nonmalignant epithelial cell differentiation characteristics will have been retained by the tumour cells depending on the position of the differentiation block. The initial genetic hit(s) underlying the malignancy will have occurred in the presumably estrogen-dependent undifferentiated epithelial cell population of the breast.[4] However, additional genetic alterations are likely to be needed to establish a full breast malignancy[20,145] and these may have occurred at later differentiation stages. Some of these molecular changes will have disrupted terminal cell differentiation, resulting in deregulated cell proliferation and apoptosis. An example of contrasting cell differentiation properties retained in breast tumours is presented by the presence or absence of ERα.

While all breast tumours are derived from the estrogen-dependent epithelial precursor cell population, one-third of carcinomas are ERα-negative but do express the EGF receptor.[53,146,147] The majority of breast carcinomas are ERα-positive growing tumours, but the corresponding nonmalignant, ERα-positive luminal epithelial cells do not proliferate.[148-150] Some evidence exists for low numbers of ERα-positive/EGFR-positive normal breast epithelial cells,[146] which may represent an intermediate differentiation stage. The corresponding malignant counterpart of these double-positive cells appears extremely rare, in agreement with the observation that efficient tumour cell proliferation is not compatible with simultaneous activation of both signalling pathways.[109] Similarly, it may be envisaged that the retained differentiation stage-specific growth regulatory pathways will determine the tumour response to tamoxifen treatment. The availability of an alternative driving force – independent of ERα – will facilitate the survival and proliferation of tumour cells during tamoxifen-induced stress conditions.

5.2 Apoptosis and Breast Epithelial Cell Differentiation

Another unresolved aspect of breast carcinomas is their sensitivity for apoptotic signals. Apoptosis is an essential part of breast physiology during menstrual cycles and pregnancy, and after termination of lactation.[151] However, not all epithelial (precursor) cells are removed during these phases and specificity in apoptosis must be imprinted in the particular cell differentiation programme. Although evasion of apoptosis is considered to be an essential step in tumour development,[20] residual sensitivity to apoptotic signals can be a decisive factor for a favourable therapeutic effect. A significant proportion of the estrogen-dependent breast cancers are growth-arrested by antiestrogen treatment, but their elimination will depend on the activity of the apoptotic pathway.[152,153]

In the absence of a functional apoptotic pathway, only stable disease may be achieved during tamoxifen treatment (table I). Tumour cells with strong potential for apoptosis may respond to the antiestrogen-mediated growth arrest by (partial) disappearance (partial or complete tumour remission). The similar survival characteristics of patients with long term stable disease (>6 months) or with partial remission[45-47] suggest that tumour cell proliferation control by tamoxifen is more important for patient survival than apoptosis.

5.3 Antiestrogen Resistance and Breast Epithelial Cell Differentiation

The aspects of growth control and apoptosis mentioned in sections 5.1 and 5.2, which are part of normal breast epithelial cell differentiation, may explain the variable initial response of the tumour depending on the properties of the malignant cells (table I). But can it contribute to our understanding of acquired tamoxifen resistance, i.e. the progression of the disease after initial response? It should be kept in mind that after initial response, disease

Table I. Breast cancer response to tamoxifen

Progressive disease is caused by a lack of inhibitory effect of tamoxifen caused by the presence of an efficient growth control pathway independent of estrogen signalling
Stable disease is the consequence of an efficient blockade of the estrogen signalling pathway and the absence of an efficient alternative pathway of growth control. Apoptosis does not contribute strongly in these patients
Partial and complete remissions are the result of efficient abrogation of the estrogen-induced growth and the (strong) sensitivity of these tumour cells to growth-arrest–induced apoptosis

progression is scored following tumour growth or appearance at any site. For complete and partial remissions, the site of progression may be distinct from the original sites used to determine the response. As a consequence, disease progression in many patients may represent growth of another tumour clone which appeared (much) later and may not have responded to treatment in the same favourable fashion as the diagnosed metastasis. Similar observations have already been made for discrepant ER expression between primary breast tumours and their metastatic lesions.[154,155]

Thus, the group of tumours with acquired tamoxifen resistance may be smaller and less homogeneous than generally accepted. Yet it is clear that, at least in some patients, the tumour adapts to the treatment and progresses into a resistant phenotype. Slight alterations in expression of important (co-)regulatory genes capable of supporting a weak alternative growth pathway or causing reduction of apoptosis may then allow for tumour regrowth. These induced changes may affect the same pathways that are involved in intrinsic tamoxifen resistance. The observation that acquired tamoxifen-resistant tumours respond better to second-line endocrine treatment targeted at ER than intrinsically resistant tumours[61,62] suggests that in these patients the estrogen signalling pathway has remained at least partially active. Application of a different endocrine treatment may nullify the tamoxifen treatment-induced epigenetic changes and may cause a secondary tumour response. But again, this response is mostly short lived and tumour regrowth will occur.

6. Future

The response to tamoxifen and other endocrine treatment regimens appears to be dictated by the differentiation properties – i.e. proliferation control and apoptosis – retained by the tumour cells. Additional information on the growth control programmes operational in normal breast epithelial cells and in malignant cells is needed to interfere efficiently with tumour growth.

Application of micro-array gene expression profiling[156,157] will provide much more information on the differences in gene expression patterns of breast tumours, which so far by standard diagnostic criteria appear to be rather similar. Novel information on tumour classification has already been obtained for B cell lymphoma, leukaemia and melanoma.[158-160] In addition, suggestions for alternative classification of breast carcinomas have been obtained using gene expression profiling.[105,106,161] By comparing the gene expression profiles of the antiestrogen-resistant cell line models and a large number of selected specimens of breast carcinoma with a documented type of response to tamoxifen treatment, we may gather detailed insight into the mechanism of tumour response to endocrine manipulation. This information may result in improved disease diagnosis and therapy selection, and ultimately in development of targeted combination therapies aimed at preserving antiestrogen-mediated tumour control.

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