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THE BIOLOGY OF BREAST TUMOR PROGRESSION

Acquisition of hormone independence and resistance to cytotoxic drugs

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Many breast tumors appear to follow a predictable clinical pattern, being initially responsive to endocrine therapy and to cytotoxic chemotherapy but ultimately exhibiting a phenotype resistant to both modalities. Using the MCF-7 human breast cancer cell line as an example of an 'early' phenotype (estrogen and progesterone receptor positive, steroid responsive, low metastatic potential), we have isolated and characterized a series of hormone-independent but hormone-responsive variants (MIII and MCF7/LCC1). However, these variants remain responsive to both antiestrogens and cytotoxic drugs (methotrexate and colchicine). MIII and MCF7/LCC1 cells appear to mimic some of the critical aspects of the early progression to a more aggressive phenotype. An examination of the phenotype of these cells suggests that some hormone-independent breast cancer cells are derived from hormone-dependent parental cells. The development of a hormone-independent phenotype can arise independently of acquisition of a cytotoxic drug resistant phenotype.

Key words: Breast cancer, progression, cell biology.

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Breast cancer is the most common form of cancer in women living in western societies. Approximately 10% of all women in the USA and western Europe living to age 80 will develop breast cancer. The incidence of breast cancer is inexorably increasing, with an annual worldwide incidence of over one million predicted by the turn of this century (1). A major problem in breast cancer therapy is the almost inevitable progression from hormone-dependent to hormone-independent growth. Over 30% of all human breast tumors expressing both estrogen receptors (ER) and progesterone receptors (PGR) fail to regress following antiestrogen treatment (2, 3). Thus, loss of hormone-dependence is a critical step in the development of breast cancer.

Following therapeutic intervention with cytotoxic chemotherapy, many breast tumors appear to undergo an objective remission. However, tumors ultimately exhibit a multi-drug resistant phenotype. Whilst overexpression of the MDR1 gene may contribute to drug resistance (4–6), other mechanisms of resistance are clearly important. The development of multiple metastatic lesions which are resistant to therapy is the major cause of death in cancer patients (7). If breast tumors did not metastasize, a high proportion of all patients could be cured by local therapies. These observations define a loss of hormone-dependence and the acquisition of a metastatic phenotype which is resistant to currently available cytotoxic chemotherapy as being the most critical biological properties of breast tumors.

The postreceptor binding mechanisms through which steroid hormones elicit mitogenic or biological responses in target tissues are unknown but clearly must involve alterations in gene expression. From the perspective of breast

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cancer, the induction of PGR synthesis following stimulation with estradiol is used as an indicator of a functional receptor system. Tumors expressing both ER and PGR exhibit a greater response rate to antihormonal therapy than tumors expressing neither of these receptors or either receptor alone (2, 8). Other E2-induced genes including pS2 also have prognostic significance (9). Whilst fulfilling an important practical function, induction of PGR or pS2 synthesis is unlikely to mediate the mitogenic effects of estradiol. Consequently, considerable effort has been expended in determining the ability of steroid hormones to regulate the expression of genes associated with cellular proliferation.

De Larco & Todaro (10) have suggested that some tumor cells may produce the factors they require for continued proliferation. These factors could subsequently function in an autostimulatory (autocrine) manner. For hormone-dependent breast cancer, identification of these factors has involved investigating steroid-induced alterations in the level of expression of mitogenic growth factors, inhibitory growth factors and oncogenes (11). Breast cancer cells possess receptors for a number of mitogenic growth factors and also secrete significant amounts of the appropriate ligands for these growth factor receptors. Hence, an autocrine stimulatory mechanism may control breast tumor proliferation. This autocrine loop could function externally, where the cells would secrete the ligand which could then bind to its receptor on the surface of the same cell from which it was secreted (11). An internal autocrine loop may also function with the ligand-receptor interactions occurring intracellularly, perhaps at the endoplasmic reticulum-Golgi complexes or within secretory vesicles (12).

For hormone-dependent breast cancer cells, the autocrine hypothesis would predict that the levels of mitogenic growth factors produced would increase and inhibitory growth factors would decrease on stimulation with estradiol. Exposure to antiestrogens might increase the production of inhibitory factors, whilst decreasing production of mitogens. The ability of estrogen to regulate the secretion of a number of growth factors have been reported. These include the mitogens epidermal growth factor (EGF), transforming growth factor-alpha (TGF-α) and other TGF-α-like proteins, the insulin-like growth factors (IGF) and fibroblast growth factors. Direct evidence for a role of these estrogen-stimulated factors was obtained by Dickson et al. (13) who observed the ability of conditioned cell culture medium obtained from MCF-7 cells growing in vitro to partly support the transient growth of these estrogen-dependent cells in castrated athymic nude mice.

In contrast, hormone-independent cells would be predicted to constitutively express high levels of mitogenic ligands in the absence of hormone and would not respond to hormonal stimulation. Hormone-independent cell lines frequently exhibit a high level of constitutive growth factor expression which is not hormone-regulated (14, 15). This ability to secrete high amounts of mitogenic autocrine growth factors may be responsible for hormone-autonomous growth (11, 15). The validity of the autocrine hypothesis remains unclear. We have observed that the constitutive expression of TGF-α alone is insufficient to induce the hormone-independent phenotype in MCF-7 cells (16). Transfection with other hormone-regulated growth factors/receptors and oncogenes including EGF-R (17) and *c-myc* (18) have also failed to induce hormone-independence in MCF-7 cells.

Whilst there is considerable indirect evidence to support the autocrine hypothesis, the factor or factors which can confer hormone-independence remain to be elucidated. The prognostic significance of E2-regulated genes such as pS2 or PGR indicate that the presence of a functional ER system has significant implications for the biology of breast tumors, by indicating the hormone-responsive/dependent nature of the cells. Unfortunately, the precise relationship between initially sensitive populations and their resistant descendant populations remain unclear. This largely reflects the absence of appropriate cellular models for the generation and experimental testing of relevant hypotheses. We shall describe the isolation and characterization of a series of hormone-independent human breast cancer cell line variants which we believe will enable us begin to address some of these inter-relationships and the molecular mechanisms which confer critical biological properties upon some breast tumors.

The malignant progression of breast cancer and its implications for breast tumor biology

There is considerable interest in the identification of the cell types from which breast tumors arise. However, despite substantial effort, the precise nature of the progenitors of most human breast tumors remains to be determined. By examining the biological characteristics of putative risk factors for the development of breast cancer, and the histological characteristics of some benign and malignant tumors, it may be possible to infer some properties of the early neoplastic phenotype.

The relationship between benign breast lesions and breast cancer remains an area of intense interest and considerable speculation. Whilst a history of benign breast disease is a risk factor for developing breast cancer, the majority of women with benign breast disease never develop malignant disease. Some women who are diagnosed with a malignant breast tumor also present with concurrent benign lesions (19–21). Not all in situ carcinomas necessarily progress to invasive disease (22), and not all invasive carcinomas have metastasized by the time of diagnosis (23). However, the biological relationship between these in situ tumors and the associated invasive,

Table 1

Potential risk factors for the development of breast cancer which appear to have an endocrine-related mechanism

Female sex (33)
Primary ovarian failure (30)
Early menarche and/or late menopause (29, 30)
High intake of dietary fat (1)
Obesity (31, 32)
Late first full-term pregnancy (30, 36).
Nulliparity (30)
Elevated serum estrogens/lower sex hormone binding globulin (29, 34).

metastatic or benign disease remains to be elucidated. However, we can infer some potential characteristics of the early malignant phenotype. The common phenotypic characteristics between benign tumors and carcinomas in situ are their ductal or intraductal location and the absence of evidence of local invasion into surrounding stromal tissues. Thus, the location of the early neoplastic breast cell populations appear to arise from the cells lining the terminal ductal-lobular unit (24). These structures contain both epithelial and myoepithelial cells. Since tumors of apparently myoepithelial origin occur very rarely in the human breast (25, 26), and myoepithelial cells are frequently absent from breast tumors (27), the epithelial rather than myoepithelial derivation of tumors is clearly indicated. The pathological changes associated with the early stages of breast tumor development and their implications have been extensively reviewed by Russo et al. (28). The presence of in situ tumors, and the observation that not all of these necessarily become invasive (22), suggests that the appearance of a high metastatic/invasive potential does not necessarily develop concurrent with the initial appearance of neoplastic foci.

Many risk factors for breast cancer appear to exhibit a hormonal mechanism (Table 1). For example, the effect of early menarche (29) and late menopause would be to increase the time of exposure of breast tissue to estrogenic stimulation (30). Similarly, nulliparity maintains a high degree of non-terminally differentiated stem breast cells which are exposed to estrogenic stimulation (30). Pregnancy and prolonged lactation involve the differentiation of breast epithelium. Thus, early first full-term pregnancy and breast feeding appear to have some protective effects, perhaps by inducing terminal differentiation of breast stem cells (30). High dietary fat may be associated with obesity. Aromatization of steroid precursors to 17β-estradiol occurs predominantly in adipose tissue in postmenopausal women. Therefore, these two risk factors may function through an induction of increased circulating estrogen levels (31, 32). Of relevance is the observation that either primary ovarian failure, or ovariectomy at an early age, can reduce the risk of developing breast cancer (30). The greatest risk of breast cancer is female sex. The incidence of breast tumors in men is 1% of that observed in women (33). Elevated serum estrogen concentrations and lower sex hormone binding globulin levels are also closely associated with an elevated risk of breast cancer (29, 34). For these endocrine-based explanations of the mechanisms of the risk factors to be biologically relevant, we infer that the breast tumor precursor cell populations are probably estrogen-responsive and, therefore, ER positive.

In normal breast epithelium mitoses peak in the luteal phase of the menstrual cycle, during which time progesterone levels are at their highest and estrogen at their lowest. Some investigators have suggested that progesterone plays a pivotal role in mitosis (35). Whilst the role of progesterone requires further study, one hypothesis of hormonal carcinogenesis in breast cancer invokes the 'total cumulative exposure of breast tissue to bioavailable estrogens and the associated cumulative mitotic activity' as the important etiological factors (36). This hypothesis implies that the early preneoplastic cells would express functional receptors for progesterone (PGR) and/or ER. The hormone-responsivity of a significant proportion of human breast tumors (3) indicates that cells expressing this phenotype can persist throughout the biological progression of the tumor. Thus, we conclude that a hormone-dependent or hormone-responsive phenotype is not necessarily disadvantageous to the cell.

These observations suggest that one putative 'early' phenotype of a breast cancer cell would result from the neoplastic conversion of an ER and PGR positive, steroid responsive, ductal or intraductal epithelial cell. Potential candidates for these hypothetical neoplastic precursor cells can be found in normal breast tissue. ER positive epithelial cells in normal mature human breast tissues have been reported (37–39). These ER positive epithelial cells occur as single cells and are more prevalent in the lobular structures than the interlobular ducts (38). In normal breast, these potentially hormone-responsive cells may be responsible for the proliferative and secretory functions associated with pregnancy and lactation.

Early neoplastic populations would be non-invasive (and by implication non-metastatic). The application of selective pressures (e.g. immunological, nutrient deprivation, therapeutic intervention) could, over a period of time, induce the apparent progression of these cells towards an invasive, metastatic, hormone and cytotoxic drug resistant tumor (40). Despite the probable monoclonal origin of breast tumors, they develop marked subpopulation heterogeneity (41, 42). Thus, in a manner analogous to the evolution of subspecies, some tumor cell populations produce the appearance of adapting to selective pressures. The association between increasing tumor size and increasing aggressivity (43), and the potentially long (apparent) doubling time of primary human breast tumors (44, 45), provide further indirect evidence in support of this hypothesis. Both of these observations probably reflect the greater

number of cell divisions since transformation required to produce the larger tumors. Clearly the larger the tumor population, the greater the probability that there will be cells which have acquired the necessary phenotypes for increased malignancy. Tumors are constantly losing reproductively potent cells with appropriate phenotypes as a result of their terminal differentiation, mutation to disadvantageous phenotypes, loss to other compartments (e.g. local invasion and metastasis) and cell death. The long apparent doubling time may be the result of more than the minimum 30 doublings generally considered necessary for a single tumor cell to produce a clinically detectable mass. Thus, the greater the potential for the generation of variants with a biological advantage.

Models for progression in breast cancer

Administration of carcinogens such as 7,12-dimethylbenz(a)anthracene (DMBA) or N-nitrosomethylurea (NMU) can induce mammary tumors in rodents. These models have provided considerable insight into the basic properties of breast tumors and the processes of carcinogenesis and tumor promotion, reflecting the biological similarities between these and naturally arising mammary tumors. However, there are a few important exceptions. Experimental rodent tumors frequently exhibit considerable variations in intertumor growth characteristics (46), exhibit a well-differentiated morphology and a low metastatic potential (47). These tumors exhibit a rapid progression to hormone-independent growth (48, 49) and a complete initial dependence upon prolactin (47, 50). To date, no such central role for prolactin has been clearly attributed to human breast cancers (51). Approximately 75% of rodent mammary tumors induced by NMU and 20% induced by DMBA (52) exhibit a high incidence of either altered or activated *ras* expression, which apparently occurs during initiation (53, 54). The few metastases that do arise from these chemically-induced tumors have been attributed to these perturbations in *ras* expression (55). However, altered or mutated *ras* expression appears to occur infrequently in human breast cancer (54–56).

A number of human breast cancer cell lines have been established in vitro. The utilization of these cells growing as tumors in athymic nude mice has proved a powerful model for the study of human tumor biology. The ease of both in vitro and in vivo maintenance, the human derivation of the tissue, and the similarities in plasma estrogen levels between nude mice and postmenopausal women (57, 58) are significant advantages. The majority of ER positive lines are fully hormone-dependent and frequently exhibit a poor metastatic potential. The hormone-dependent cell lines most highly characterized are MCF-7, ZR-75-1 and T47D. There is no evidence of significant metastatic or invasive spread from ZR-75-1 or T47D cells. Two early reports described the presence of neoplastic lung deposits arising from MCF-7 tumors with frequencies of 15% (59) up to 40% (60). Tumor growth and metastasis was fully E2-dependent (59, 60). In marked contrast, the majority of investigators have failed to observe significant secondary lung deposits from these cells (16, 57, 61, 62). The generation of metastases from primary human breast tumor cells growing in nude mice is also very rare (57, 63, 64). Whilst there is considerable disagreement regarding the metastatic potential of MCF-7 cells, the majority of investigators appear to be in agreement regarding the poor invasive capability and E2-dependence of MCF-7 cells, regardless of the presence of metastases (16, 57, 59, 61, 62).

There are more ER negative than ER positive human breast cancer cell lines. These ER negative cell lines will form proliferating tumors in the presence or absence of estrogen supplementation, and do not respond to estrogenic stimulation or antiestrogenic inhibition. Most of the cell lines are also poorly metastatic, although MDA-MB-231 and MDA-MB-435 cells can produce hematogenous metastases in some mice (65). These ER negative cell lines tend to represent a phenotype more characteristic of the 'late' phenotype (40).

The ability of cells to degrade/invade basement membranes is a critical component of metastasis (66). The invasive potential of cells can be examined in vitro using a reconstituted basement membrane extract (matrigel) obtained from the murine EHS tumor (67). These techniques include the Boyden chamber chemoinvasion assay (68) and the matrigel outgrowth assay (68, 69). We have analyzed several established human breast cancer cell lines in these assays, and find that they fall into two distinct groups of activities. Cells expressing vimentin (all of which are ER-negative) exhibit high activity, while those without vimentin expression irrespective of ER are poorly invasive or inactive (70). Vimentin expression is usually characteristic of mesenchymal rather than epithelial cell phenotypes, and has been found to associate with lack of ER, high growth fraction and poor nuclear grade in human breast cancer (71–73). When inoculated into nude mice, local invasiveness or hematogenous dissemination is apparent over a 60-day time period only in the vimentin positive group (70). These observations may explain, at least in part, the poorer prognosis associated wtih lack of ER and expression of vimentin in human breast cancer. The hormone-independent but hormone-responsive MIII and MCF7/LCC1 cells, whilst exhibiting an intermediate phenotype with respect to their invasive capacity, do not express vimentin. This reflects their retention of ER expression. Acquisition of vimetin expression may be more closely associted with the acquisition of a highly invasive/metastatic phenotype, and occur subsequent to a loss of ER during the process of malignant progression.

A major obstacle in the study of human breast cancer progression from hormone-dependent growth to hormone-independent growth has been the lack of suitable model

systems. There is no direct biological relationship between the ER positive and ER negative cell lines other than their human breast origin. For example, these cell lines were all derived from different women, at different times and in some cases from different sites. Almost all the breast cancer cell lines have been derived from metastatic deposits and not from primary breast lesions. Thus, there is no strong direct experimental evidence that ER negative, hormone-independent and unresponsive cells in either primary or metastatic disease are derived from ER positive, hormone-dependent cells in primary human breast tumors.

Acquisition of hormone-independence in breast cancer cells

We wished to directly address the hypothesis that the development of hormone-independence is the result of a loss of hormone-dependence by initially hormone-dependent cells. This hypothesis is supported by the apparent monoclonal origin of most solid tumors and the ultimate heterogeneity of breast neoplasms (41, 42). The total hormone-dependence, sensitivity to antiestrogens and cytotoxic drugs, and poor local invasive and metastatic capacities of MCF-7 cells, clearly indicate that these cells are representative of the putative 'early' breast cancer phenotype (40). Consequently, we wished to determine if we could isolate a hormone-independent subline of MCF-7 cells by applying a physiologically relevant selective pressure.

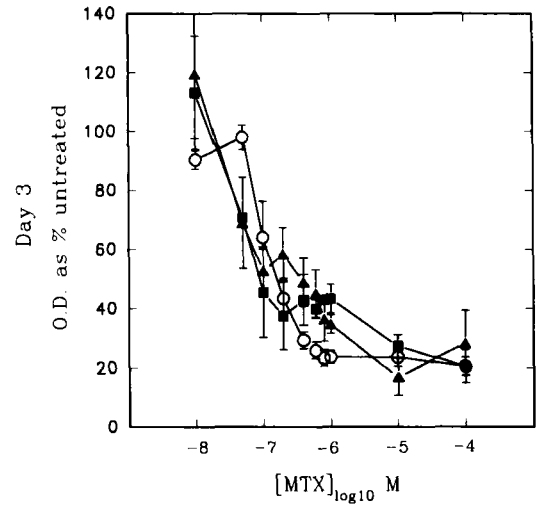
The steroid hormone levels in ovariectomized athymic nude mice are equivalent to those detected in postmenopausal women (57, 58) and MCF-7 cells were originally isolated from a postmenopausal breast cancer patient (74). Therefore, we selected MCF-7 cells following prolonged growth in the mammary fat pads of ovariectomized athymic NCr *nu/nu* mice (75, 76). A cell population designated MIII was isolated and characterized. These cells retain ER and PGR expression, do not exhibit increased EGF-R expression (76), are sensitive to inhibition with triphenylethylene, benzothiophene, steroidal and other non-steroidal antiestrogens (77) and have acquired the ability to form proliferating estrogen-responsive tumors in ovariectomized athymic nude mice (75–77). There is also evidence that MIII cells have an increased metastatic potential, as evidenced by increased basement membrane invasiveness in vitro and ability to invade into the peritoneal cavity from a mammary fat pad tumor (76, 77). We believe that these cells represent an intermediate hormone-independent but hormone-responsive phenotype with an increased metastatic potential, between the hormone-dependent, poorly invasive/metastatic and hormone-independent and the hormone-unresponsive, highly invasive/metastatic phenotypes (40).

We have now determined whether this selection has altered sensitivity to cytotoxic drugs. MIII cells were further selected by one additional passage through ovariectomized nude mice and a variant designated MCF7/LCC1 derived (78). The sensitivity of these cells to two cytotoxic drugs was determined. Since overexpression of the MDR1 gene has been implicated in the development of drug resistance in human breast cancer (4–6), we determined sensitivity to the antimetabolite methotrexate (MTX), a drug which is not a substrate for MDR1, and to the mitotic inhibitor colchicine (Colch) which is a classic MDR1 substrate.

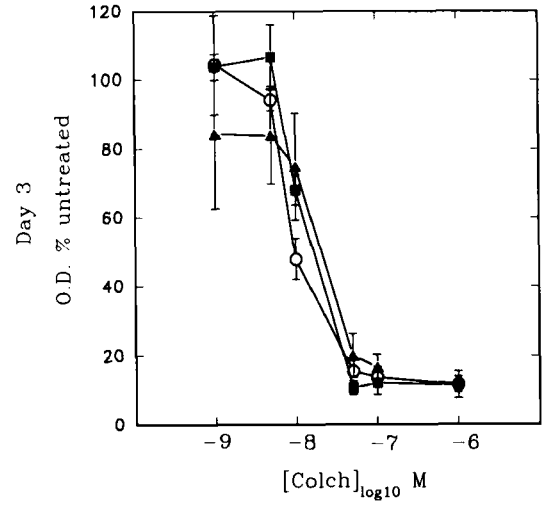
Cells were seeded into 96 well tissue culture dishes and exposed to clinically relevant concentrations of MTX as determined by utilizing a minimum plasma a.u.c. value and estimating the dose range such that the $C \times t$ value produced an equivalent dose following a 24-h incubation in vitro (79). Cytotoxicity was determined using a crystal violet dye uptake assay 72 h after removal of the drug. The degree of dye uptake is directly related to cell number. Briefly, cells are stained with the crystal violet stain by incubation with staining solution (0.5% (w/v) crystal violet in 25% (v/v) methanol) for 5 min at 25°C and rinsing gently twice with distilled H_2O. Cells are allowed to dry and the dye extracted by the addition of 0.1 M sodium citrate in 50% (v/v) ethanol and incubating at room temperature for 10–15 min. Absorbance is read at 540 nm using a Dynatech MR700 ELISA reader (Dynatech, Chantilly, VA, USA). The data are presented as the mean and SD of four determinations and represent the optical density expressed as a percentage of untreated cell populations.

The preliminary data presented in Fig. 1a and b clearly indicate that the sensitivity of parental MCF-7 cells and the hormone-independent variant MCF7/LCC1 are equivalent to both MTX and Colch. Thus, selection for hormone-independence does not alter sensitivity to cytotoxic chemotherapy. The equivalent sensitivity to both a MDR1 substrate (Colch) and a non-substrate (MTX) also implies that these cells have not induced an overexpression of functional MDR1 protein. We have previously demonstrated the ability of estrogens to increase the cytotoxicity of MTX in the parental MCF-7 cells, probably as a result of estrogen to increase the rate of DNA synthesis and cell proliferation (80). Whilst the phenol red used in cell culture medium contains significant estrogenic activity (81), it is unable to influence the sensitivity of MCF7/LCC1 cells to MTX. This reflects the inability of MCF7/LCC1 and other hormone-independent variants to respond mitogenically to estrogenic stimuli in vitro (76, 77). These observations may have important implications for the use of estrogenic recruitment in some chemohormonal regimens (for recent review see (15)).

A comparison of the phenotypes of the hormone-independent variants has enabled us to better understand the interrelationship of some of the characteristics associated with the process of malignant progression in human breast cancer. These comparisons strongly suggest that the factors contributing to perturbations in antiestrogen and cytotoxic drug sensitivities, hormone-dependent growth, metastatic potential and tumorigenicity are essentially



(a)



(b)

Fig. 1. The response of hormone-dependent MCF-7 cells and the hormone-independent variant MCF7/LCC1 to methotrexate (1a) and colchicine (1b). Cells were seeded into 96 well tissue culture dishes and exposed to clinically relevant concentrations of drug for 24 h. Cytotoxicity was determined using a crystal violet dye assay 72 h after removal of the drug as described in the text. The data presented are the mean and SD of 4 determinations and represent the optical density expressed as a percentage of untreated cell populations. MCF7/LCC1 without phenol red [▲-▲]; MCF7/LCC1 cells with phenol red [■-■]; MCF-7 cells [○-○].

independent of each other. Loss of ER expression and overexpression of EGF-R probably occur later in the process of malignant progression. A more detailed discussion of these observations and their implications is provided elsewhere (40).

Mechanisms for acquisition of hormone-independent growth in breast cancer cells

We wished to determine the association of perturbations in the regulation of gene expression with progression to a more malignant phenotype. The potential mechanisms which could contribute to the progression of human breast tumors are indicated in Table 2. These include gene-amplifications which are associated with a more malignant phenotype (82, 83), oncogene overexpression, growth factor production (15), or increased in steroid biosynthesis. We also hypothesized the perturbations in the expression of specific E2-regulated genes, may be associated with a loss of hormone-dependence. Table 3 lists a series of estrogen-regulated genes which could either contribute directly to malignant progression, or function as markers for altered hormone-regulation of gene expression.

Preliminary data indicate that the amplification of genomic sequences is not associated with progression (78). The dose–response curves of MCF-7, MIII and MCF7/LCC1 cells to the aromatase inhibitor 4-hydroxyandrostenedione are essentially identical (R. Clarke, unpublished observations), indicating that increased steroid biosynthesis is unlikely. The altered expression of specific genes may confer some characteristics of the progressed phenotype ((78) and Brünner et al. manuscript submitted). For example, there appears to be an increase in the expression of PGR and pS2 (76, 78) but not ER (76). This overexpression of PGR/pS2 is unlikely to be mechanistically responsible for the acquisition of hormone-independence. However, the alterations in the pattern of hormone-regulation provide

Table 2

Potential mechanisms responsible for the malignant progression of human breast tumors

1. Amplification of specific genes.
2. Activation or overexpression of specific oncogenes or proto-oncogenes e.g. myc, c-erbB-2 etc.
3. Increased production or sensitivity to secreted mitogenic factors e.g. TGF-α, IGF-I, IGF-II etc.
4. Decreased production or sensitivity to inhibitory factors e.g. TGF-β.
5. Increased steroid biosynthesis (aromatase or sulfatase activities) by the neoplastic cells.

Table 3

Estrogen-regulated genes. The altered expression of these and other hormone-regulated genes may indicate the role of perturbations in gene expression in the acquisition of hormone-independent growth. Citations describe the hormonal regulation of the listed genes

1. Estrogen receptor (ref. 85)
2. Progesterone receptor (ref. 86)
3. pS2 (ref. 9)
4. 52 k (cathepsin D) (ref. 87)
5. TGF-alpha (ref. 14).
6. IGF-I, IGF-II (ref. 88)
7. TGF-beta (ref. 89)

direct evidence in support of our hypothesis that acquisition of hormone-independence is associated with the altered expression of specific hormone-regulated genes. We are currently in the process of identifying the genes which are differentially expressed in these hormone-dependent and hormone-independent MCF-7 variants.

Discussion

Many breast tumors appear to follow a remarkably predictable pattern of tumor progression. Tumors which are locally confined (e.g. ductal carcinoma in situ with no evidence of axillary lymph node involvement) are often curable by local therapy such as mastectomy with or without axillary lymph node irradiation. A high proportion of breast tumors are sensitive to endocrine manipulation and cytotoxic chemotherapy. However, following therapeutic intervention with antiestrogen therapy and cytotoxic chemotherapy, many of these tumors acquire a phenotype characterized by resistance to both therapies. The high frequency of the development of drug resistance is evidenced by the limited increases in overall survival in patients who have received these therapies (84). The resultant tumors may represent the presence of pre-existing resistant tumor populations or the adaptation of subpopulations to these therapeutic selective pressures. The high frequency of this progression strongly suggests that it is an inherent property of many breast cancer cells. We consider that there are at least three critical steps in this process of progression towards a highly aggressive phenotype. These stages are the acquisition of hormone-independence, increased invasive/metastatic potential, and the acquisition of a multidrug resistant phenotype (not necessarily involving MDR1).

We have suggested a hypothetical 'early' breast cancer cell phenotype which is ER and PGR positive, steroid responsive, non-invasive (and by implication non-metastatic) of either ductal or intraductal epithelial origin. Using the MCF-7 cell line as an example of cells which represent many of these characteristics, we have isolated and characterized a series of hormone-independent but hormone-responsive variants. We believe that these cells provide a unique model to study the progression of hormone-dependent breast tumors in postmenopausal women. A comparison of the phenotype of these cells supports our hypothesis that hormone-independent cells are derived from hormone-dependent parental cells. The mechanisms which confer this hormone-independence include the altered expression of specific hormone-regulated genes. The development of novel therapeutic strategies for breast cancer clearly require a more detailed understanding of the mechanisms involved in cellular transformation, progression and promotion. We hope that the cellular models we have isolated will prove useful in the generation and testing of novel hypotheses, and for the isolation of genes associated with the process of malignant progression in human breast cancer.

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