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Acquired endocrine resistance in breast cancer: implications for tumour metastasis

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1. ABSTRACT

Endocrine therapy is the treatment of choice in hormone receptor-positive breast cancer. However, the effectiveness of these agents is limited by the development of drug resistance, ultimately leading to disease progression and patient mortality. Whilst pre-clinical cell models of acquired endocrine resistance have demonstrated a role for altered growth factor signalling in the development of an endocrine insensitive phenotype, it is becoming apparent that acquisition of endocrine resistance in breast cancer is also accompanied by the development of an adverse cellular phenotype, with resistant cells exhibiting altered adhesive interactions, enhanced migratory and invasive behaviour, and a capacity to induce angiogenic responses in endothelium. Since invasion and metastasis of cancer cells is a major cause of mortality in breast cancer patients, elucidation of molecular mechanisms underlying the adverse cellular features that accompany acquired endocrine resistance and their subsequent targeting may provide a means of limiting the progression of such tumours *in vivo*.

2. INTRODUCTION

Breast cancer is the most common form of malignancy experienced by women, with a lifetime risk of contracting this disease of around 1 in 9. Breast cancer is second only to lung cancer as the most common form of cancer-related death in the USA although encouragingly, mortality rates are decreasing due to early detection and ever improving treatment regimens. The principal cause of death in breast cancer, as with other cancers, is the presence of metastatic disease. The propensity for tumours to metastasize has been known since the first description of a metastatic breast tumour located in the brain (1). Importantly, subsequent observations by Paget reported in 1889 revealed that metastatic tumours, rather than being capriciously distributed within the body, generally exhibited a tissue-specific pattern of distribution according to the tumour from which they arose. Breast cancers display a propensity to metastasise to particular organs, primarily the bone, liver, lung and brain. Indeed, the skeleton is the site of distant relapse in almost 50% of cases (2). Furthermore, with the knowledge that breast cancer

Table 1. Steps of the metastatic cascade

Modulation of cell-cell adhesion within tumour
Disruption of basement membrane
Cell migration
Invasion into host lymphatic/blood system (intravasation)
Survival within the circulatory system
Arrest at distant (metastatic) site
Extravasation
Dormancy/proliferation

now represents at least five different subtypes, these subtypes have recently been shown to display different metastatic 'preferences', i.e. different breast cancer subtypes spread preferentially to different tissues (3).

2.1 The metastatic cascade

Tumours comprise a heterogeneous collection of cancer cells, representing a spectrum of proliferative abilities, chemosensitivities and invasive potential. Only a small proportion of these cells possess the required characteristics necessary to metastasize. These 'metastatically-competent' cells frequently exhibit deregulation of numerous genes and proteins resulting in a phenotypic change that allows them to successfully proceed through a series of interrelated steps collectively termed the 'metastatic cascade' (Table 1)

Metastatic cell characteristics (for example loss of cell-cell adhesion, protease production and cytoskeletal re-organisation) may pre-exist within individual cells in the main tumour mass or may be acquired during the development of the tumour; in such cases it is clear that events which promote the acquisition of these characteristics may well play a key role in promoting tumour spread. Importantly, pre-clinical evidence now suggests that chronic exposure of breast cancer cells to a range of hormonal therapies results in acquisition of resistance to these agents and is accompanied by the development of adverse tumour cell characteristics; if recapitulated *in vivo*, acquired resistance to such therapies may thus facilitate metastatic progression. In this article, we highlight several recently-identified mechanisms which contribute to the establishment of an invasive and migratory phenotype in breast cancer cells following the acquisition of endocrine resistance. These molecules and their associated pathways, deregulated on long-term endocrine exposure, may ultimately provide novel therapeutic targets with which to circumvent resistance development and inhibit breast tumour spread.

3. ACQUIRED ENDOCRINE RESISTANCE PROMOTES METASTATIC CELL CHARACTERISTICS IN PRECLINICAL BREAST CANCER CELL MODELS

Around three-quarters of breast cancers express the oestrogen receptor-alpha and are thus potentially sensitive to the growth–promoting effects of steroid hormones. As such, endocrine therapies which seek to disrupt the steroid hormone environment of the tumour can promote extensive remissions in established cancers, providing significant benefits in patient survival (4). However, despite the undoubted improvements brought about by endocrine treatments, their clinical success is limited by the phenomenon of resistance, with more than a third of patients with endocrine-responsive, early stage breast cancer and almost all of those with metastatic disease becoming refractory to these treatments during the course of their disease (acquired resistance) and the outlook for these patients is poor. (4-6). Clinical relapse during endocrine therapy has been linked to tumours that have gained an aggressive phenotype and enhanced metastatic capacity and is frequently associated with a poorer outlook for the patient. However, little is known about the mechanism(s) that underlie such disease progression and spread and whether they are induced by drug treatment.

A great deal of research has been undertaken in order to understand the mechanisms that underlie the phenomenon of endocrine resistance with a view to revealing markers that predict for response to, or early relapse on, treatment in addition to identifying potential therapeutic targets through which endocrine resistance may be delayed or prevented. These studies have clearly demonstrated the ability of tumour cells to harness a variety of growth factor signalling pathways that can drive growth in the presence of endocrine agents. Importantly, it is now clear that endocrine agents themselves can promote the expression of both growth factor receptors and numerous ligands during the drug-responsive phase that subsequently promote tumour growth during the drug-resistant phase (7, 8). The role of growth factor signalling in endocrine resistance has thus gained significant attention over the past decade and there is now compelling evidence which suggests that the inappropriate activation of growth factor signalling cascades can readily promote anti-hormone failure in breast cancer cells. Indeed, overexpression of members of the erbB family of receptor tyrosine kinases including the epidermal growth factor receptor (EGFR), HER2, HER3 and the insulin-like growth factor-1 receptor (IGF1R) together with several of their ligands have all been suggested to play a central role in mediating an endocrine resistant state in some situations (8-12). In such cases, the enhanced expression of these growth factor signalling pathways are likely to contribute to endocrine resistance through cross-talk with the ER resulting in its ligand-independent activation which sustains cellular growth (8, 13, 14). Importantly, these growth factor signalling pathways which act to promote breast cancer cell proliferation in an endocrine-resistant context are also known also to play prominent roles as regulators of cellular migration and invasion in other cell systems (15-17) and it therefore follows that resistance to endocrine agents in breast cancer may result in the development of an adverse cellular phenotype. Indeed, evidence suggests that the acquisition of resistance to endocrine therapies is also accompanied by a significant enhancement of the cells' migratory and invasive potential *in vitro* (18-21). Clearly, these *in vitro* observations suggest that endocrine-resistant tumours possess aggressive characteristics which, *in vivo,* are likely to favour the dissemination of tumour cells from the primary tumour and thus promote disease spread. However, because inhibition of growth factor receptors shown to play a dominant role in regulating the growth of acquired resistant breast cancer cells results only in a

modest suppression of their invasive phenotype (18), it is likely that the adverse tumour cell characteristics developed following chronic exposure to endocrine therapies arise due to alterations in other mechanisms independent of these growth factor receptors previously described. The ability to identify the dominant pro-invasive/migratory mechanisms activated as a consequence of endocrine treatment may ultimately aid in the development of therapies which may prove central to the successful treatment of aggressive disease associated with relapse on endocrine therapies and improve prognosis as a consequence. To this end, we and others have recently described a number of molecules and pathways which are activated in an endocrine-resistant state and act to promote aggressive cellular phenotype *in vitro*.

3.1. Modulation of tumour cell adhesion in acquired endocrine resistance

In their natural environment, cells are in constant contact with protein components of the extracellular matrix and generally with other cells of the same or different type. These adhesive interactions are maintained through a diverse array of cell surface adhesion receptors and allow the cell to sense the microenvironment in which it resides and respond accordingly. These adhesive interactions provide platforms to orchestrate cell shape changes and activation of signalling pathways downstream of adhesion receptors facilitate cell survival and growth in addition to promoting migration upon appropriate stimulus. Coordinated regulation of homotypic cell adhesion plays a key role in epithelial to mesenchymal transitions (EMT), a state where cell adhesion is reduced while migration is stimulated and is central to both physiological responses (e.g. during embryogenesis and wound repair) and pathological states (e.g. during tumour metastasis).

3.1.1. Cell-cell adhesion

Pre-clinical models of acquired endocrine-resistant breast cancer commonly show a more angular, dedifferentiated morphology with numerous lamellipodia and membrane ruffling in addition to growing as loose, disorganised colonies in which cells appear to have partially-dissociated cell-cell contacts (18, 19). This apparent change in epithelial cell morphology and colony integrity observed within endocrine-resistant cell cultures imply a loss in intercellular adhesion and suggesting that these cells might be undergoing epithelial-to-meshenchymal transition (EMT), a process well associated with a more aggressive cell phenotype (22).

E-cadherin plays a key role in establishment of cell-cell adhesion in epithelia and, with the exception of epithelial ovarian cancer and inflammatory breast cancer, epithelial tumours tend to lose E-cadherin partially or completely as they progress toward malignancy (see (23) for a review). Despite the observed morphologic differences between endocrine-sensitive and endocrine–resistant cells *in vitro*, the overall level of expression of E-cadherin in the resistant models does not appear to be significantly altered compared to their endocrine-sensitive counterparts (19, 24). However, this does not rule out the potential for E-cadherin mislocalization (24) or alterations in the expression and/or activity of its intracellular binding partners. In the latter case, we have recently observed deregulated beta-catenin expression and activity in tamoxifen-resistant MCF7 cells, particularly with respect to the phosphorylation of beta-catenin on tyrosine (19, 25), an event reported to promote dissociation of catenin-cadherin binding (26). Indeed, E-cadherin immunoprecipitates from tamoxifen-resistant cells contain much less beta-catenin than their tamoxifen-sensitive counterparts (25). These changes appear to be associated with both inactivation of GSK3-beta, via increased PI3K/AKT signalling (19), in addition to a central role for Src kinase (25, 27). Changes in the phosphorylation status of beta-catenin, in addition to regulating its binding to E-cadherin, may also promote nuclear beta-catenin localization, association with TCF/LEF-1 transcription factors and the subsequent expression of beta-catenin target genes that may further modify invasive cellular responses as has been described to occur as a consequence of deregulated Wnt signalling, of which beta-catenin is a key downstream effector of this pathway (28). Thus the consequence of beta-catenin deregulation in endocrine-resistant breast cancer cells, as well as promoting loss of cell-cell adhesion, may also extend to the expression of genes known to contribute to tumour development and spread.

3.1.2. Cell-matrix adhesion

In addition to modulations in cell-cell adhesive interactions, we have identified that acquired endocrine resistance in breast cancer cells is accompanied by a change in integrin expression (29). Consequently, the intrinsic ability of these cells to adhere to, and migrate over, components such as collagen, laminin and fibronectin are enhanced (30, 31) whilst this attachment and migration is suppressed in the presence of antibodies that neutralize the funciton of alpha-v, beta-1 and beta-6 integrins. Clearly, this has potential significance in an *in vivo* context, where adhesive interactions between tumour cells and extracellular matrix proteins are paramount to successful tumour dissemination. These observations may also have significance in lighth of the fact that integrin signalling is implicated in hormone-dependent cell proliferation. For example, high levels of alpha-5, beta-1 (fibronectin integrin) expression are detectable during periods of steroid-induced proliferation but decreased during late pregnancy and lactation and following ovariectomy (32). Thus alterations in integrin expression profile may modify the cells' response to oestrogenic signals and endocrine agents in the appropriate environment. Indeed, such effects have recently been reported in breast cancer cells where enhanced integrin expression contributes to tamoxifen resistance through a mechanism involving HER3 and Akt (33).

Engagement of integrin receptors with extracellular matrix ligands results in activation of the non-receptor tyrosine kinase, FAK (focal adhesion kinase) and the subsequent formation of focal adhesions (34). FAK is thus central to signal transduction initiated through integrin clustering (35), in addition to playing a key ole in signal transduction initiated through a number of other classes of cell-surface receptor. Activation of signalling pathways in which FAK plays a central role have been shown to be important in cell survival (36), proliferation, adhesion,

migration and invasion (37) suggesting that FAK-mediated signalling may play a key role in tumour progression and metastasis. The importance of FAK in such events has been demonstrated both *in vitro*, where expression of dominant-negative FAK mutants prevent tumour cell spreading and migration, and *in vivo*, where FAK inhibition prevents metastases to the lung of mammary cancer cells (38). Clinically, FAK levels are frequently elevated in tumour compared to normal tissue (39) and are reportedly higher in metastases compared to primary cancers (40). Overexpression of FAK in tumour tissue is associated with a poor prognosis in a number of tumour types including breast cancer (41, 42); activation of FAK in breast cancer has been shown to correlate with malignant transformation (43).

Recently, FAK activity has been demonstrated to be elevated in acquired endocrine-resistant breast cancer cell models where it appears to play a role in promoting their attachment to extracellular matrix proteins and cellular migration (44). Interestingly, this study also suggested that acquired resistance imparts sensitivity to small molecule FAK inhibitors, which effectively reduce the migratory capacity of these cells *in vitro*. Although the direct mechanism as to why FAK activity is increased in these cells remains unclear, it is likely to result from multiple cellular changes that include the aforementioned changes in integrin expression patters and growth factor pathway activity.

4. CHANGES IN CELL SURFACE RECEPTOR EXPRESSION IN ACQUIRED ENDOCRINE-RESISTANT BREAST CANCER CELLS MAY AUGMENT PARACRINE INTERACTIONS WITH THE TUMOUR MICROENVIRONMENT

It is becoming increasingly apparent that interactions between the primary tumour mass and the stroma are likely to play a central role in tumour progression. The tumour microenvironment represents a complex system in which many cell types exist, including endothelial cells, pericytes, smooth-muscle cells, fibroblasts, myofibroblasts and infiltrating immune cells, all of which can participate in tumour progression. In addition, the stromal component of the tumour harbours a multitude of factors including cytokines, chemokines and extracellular matrix proteins all able to induce signalling within the tumour cells themselves (reviewed in (45)). Indeed, given the intimate association and interplay between tumour and stroma, it is now necessary to consider the microenvironment of a cancer and its associated abnormal epithelium as a complete system rather than separate, independently-functioning compartments. The tumour microenvironment is able to both influence tumour cell proliferation and drive malignant transformation and progression of tumour cells within it, effects that occur through the paracrine action of stromal-derived growth factors on the tumour cells which themselves express the complementary receptors for these molecules and it is this reciprocal communication between cells that is frequently deregulated in aggressive cancers (46, 47).

The majority of observations on endocrine resistant breast cancer cells models derive from two-dimensional *in vitro* cultures of individual cell lines. Although these models have been demonstrated to mirror changes seen in clinical disease, they still represent relatively 'pure' experimental systems and as such do not accurately reflect the complexity of the tumour microenvironment *in vivo*. However, recent data emerging from co-culture-based systems and culture of resistant cells in the presence of exogenous ECM factors (detailed below) is beginning to reveal potential points of interplay between tumour and stroma in that endocrine resistant breast cancer cells appear to be sensitized to factors commonly found, and frequently overexpressed, within the tumour microenvironment. These observations raise the possibility that that the adverse phenotype of resistant cells may be further enhanced in an *in vivo* context.

4.1. c-Met receptor

One such case is exemplified by the c-Met receptor which we have identified as being overexpressed in fulvestrant-resistant MCF7 and T47D cells. The c-Met receptor tyrosine kinase is the cell surface receptor for hepatocyte growth factor (HGF, also known as scatter factor (SF)) and its activation results in disruption of intercellular adhesion, cell migration and invasion and promotion of angiogenesis (48). Subsequently, we have shown that co-culture of fulvestrant-resistant cells with stromal fibroblasts, known producers of HGF/SF (49), or in fibroblast-conditioned medium, results in the activation of Akt and the production of MMP2 and MMP9 and a further enhancement of these cells' invasive behaviour (50); although fibroblasts secrete a range of growth factors and cytokines that may modulate epithelial cell behaviour, our siRNA data demonstrated that these effects are specific to c-Met activation (20).

In vivo, the c-Met receptor is primarily expressed by epithelial cells and its overexpression in node-positive breast cancer identifies patients with poor clinical outcome (51). This is not surprising given the ability of c-Met to be activated in a paracrine fashion by HGF/SF-secreting stromal fibroblasts. Indeed, this mechanism has been implicated as a major contributory factor for tumour progression with studies demonstrating the ability of HGF/SF to regulate EMT and metastasis (52). Furthermore, the therapeutic value of c-Met in breast cancer has been demonstrated through studies that have used retroviral ribozyme transgenes to target HGF/SF expression in fibroblasts or the Met receptor in mammary cancer cells to inhibit paracrine stromal-tumour cell interactions (49). Since tumour invasion and spread may thus be critically influenced by paracrine influences arising from the surrounding stroma, these observations suggest that, *in vivo*, overexpression of c-Met in anti-hormone-resistant epithelial breast cancer cells may significantly affect tumour progression.

Interestingly, as well as being overexpressed in the endocrine resistant state, c-Met gene and protein expression is induced by fulvestrant in the drug-responsive phase. Such an event may act to limit the response of these

cells to fulvestrant by providing a mechanism to drive cellular growth in the absence of functional ER (induced by fulvestrant) as evidenced by out preliminary studies using fulvestrant-treated MCF7 cells (S. Hiscox, unpublished observations). An intriguing question is to how fulvestrant might modulate c-Met expression in breast cancer cells. A role for the ER is unlikely, since c-Met expression does not correlate with ER status in breast cancer tissues (51, 53). However, transcription of the c-Met gene in known to be regulated by members of the widely expressed Sp family of transcription factors (54) (55) with Sp1 activity itself influenced by ER signalling (56, 57) and thus fulvestrant treatment. Indeed, fulvestrant-induced p21Waf1 expression has been recently demonstrated in MCF7 cells through an Sp1-mediated mechanism (58). Interestingly, we have observed alterations in Sp1 and Sp3 expression in MCF7 cells on exposure to fulvestrant (S. Hiscox and N. Jordan, unpublished observations) which may thus represent one mechanism by which c-Met overexpression can be achieved.

4.2. CD44

In contrast to the overexpression of the c-Met receptor, which appears to be an effect specific to one particular endocrine agent (fulvestrant), a common feature of acquired resistance to multiple endocrine agents (tamoxifen and fulvestrant) and to oestrogen deprivation (as a model of acquired resistance to aromatase inhibitors) is the overexpression of cell surface receptors of the CD44 family (31, 59), a group of transmembrane glycoproteins implicated in the progression and spread of breast cancer. Alternative splicing and variation in glycosylation results in structural and functional diversity amongst this group of proteins (60) with several CD44 variants being associated with invasive breast cancer. For example, expression of the CD44 variant 3 (CD44v3) correlates with lymphatic spread in breast cancers (61), soluble CD44v6 is associated with lymph node metastases (62) whilst CD44v7 is associated with a reduction in disease-free survival (63). However, whilst a wealth of evidence implicates CD44 variants in tumour progression, the case for the standard form of CD44 (CD44s) is controversial. Whereas some studies report that increased expression of the CD44s correlates with patient survival (64), recent studies have demonstrated that expression of CD44s in non-metastatic MCF7 breast cancer cells promotes their migration and invasion *in vivo* (65).

In endocrine-resistant cell models, CD44s, together with the v3, v6 and v10 isoforms, are overexpressed at the gene and protein level (31). The relevance of overexpression of CD44 in these model systems has been demonstrated by siRNA knockdown experiments which reveal that loss of CD44 has an inhibitory effect on the cells' intrinsic migratory capacity *in vitro* (66-68). CD44 is also reported to associate, and form stable complexes with, a number of growth factor receptors including those of the erbB family providing a system through which cellular migration and invasion can be augmented (69, 70). This is interesting in light of our knowledge that such receptors are also overexpressed in endocrine resistance (19). Indeed, we have seen that CD44v3, and to a lesser extent CD44s, associate with the EGFR and HER2 in tamoxifen-resistant cells and the c-Met receptor in fulvestrant-resistant cells (31). The effect of this is to significantly augment the cellular invasive response to exogenous erbB ligands (in tamoxifen resistance) or HGF (in fulvestrant resistance) (31, 66, 67). A caveat to these data is that CD44 siRNA is not specific for any particular CD44 isoform but rather results in the knockdown of all forms of CD44 expressed. It is thus not possible to determine the relative contribution to the cell's aggressive phenotype from individual CD44 family members. However, it is interesting to note that examination of CD44v3 protein expression in a small series (n=77) of clinical tissue revealed an association with HER2 expression, poor survival and shortened response to endocrine therapy in ER+ patients (66, 68).

In addition to growth factors and cytokines, tumour cells are in contact with a number of extracellular matrix components in an *in vivo* situation. A number of these can act as ligands for cell surface receptors providing additional means through which the epithelial cell phenotype can be modulated. Our recent observations have revealed that activation of CD44 by hyaluronic acid (HA), an important structural component of extracellular matrices known to be concentrated in regions of high cell division and invasion (71), promotes erbB invasive signalling in tamoxifen-resistant cells (31) which may again promote an adverse cellular phenotype. Together these observations suggest that acquired resistant cells are sensitized to many factors commonly found within the tumour microenvironment such as erbB ligands, HGF/SF and the matrix components themselves. The fact that many of these factors are increased in breast cancer tissue and serum may have significant bearing on the progression of tumours following relapse on therapy.

5. ELEVATION OF SRC KINASE ACTIVITY ACCOMPANIES ACQUIRED ENDOCRINE RESISTANCE AND PROMOTES AN *IN VITRO* METASTATIC PHENOTYPE

Recently, it has become apparent that acquisition of an endocrine resistant state is accompanied by an increase in the activity of the non-receptor tyrosine kinase, Src (24, 72-75). Src interacts with a diverse array of molecules, including growth factor receptors and cell–cell adhesion receptors, integrins and steroid hormone receptors (76-79); such interactions allow Src to regulate multiple biological mechanisms important for survival, differentiation, migration and invasion in both normal and transformed cells (reviewed in (80)). In clinical breast cancer samples, elevated Src expression and/or activity has been reported in tumour tissue compared with adjacent normal tissues, where an increase in Src activity correlates with disease stage or malignant potential (reviewed in (81)). Furthermore, tumour cell lines possessing elevated Src activity are often highly metastatic, displaying an increased capacity for migration and invasion *in vitro* (82), further linking Src to tumour progression. The observations that Src activity is elevated in endocrine-resistant breast cancer cells suggest a potential causative factor for their aggressive phenotype. This is indeed the case as inhibition

of Src activity using the dual Src/Abl inhibitor, saracatinib (formerly AZD0530), abrogates invasion and migration in anti-hormone-resistant and anti-growth factor-resistant cells (72, 83).

Much evidence now demonstrates that Src may promote a migratory/invasive phenotype through its ability to modulate both cell–cell and cell–matrix adhesive interactions in tumour cells, the result of which is to promote a migratory phenotype *in vitro* which may thus favour tumour metastasis *in vivo*. Several components of the cadherin-mediated intercellular adhesion system, including ß-catenin, are direct Src substrates or are known to be downstream elements of Src-involved pathways, phosphorylated in a Src-dependent manner (84). Phosphorylation of these proteins can result in E-cadherin downregulation and/or loss of the linkage between cadherins and the cytoskeleton, promoting disruption of cell–cell contacts and contributing to increased cell migration (85). These observations may thus explain the apparent morphological changes observed in endocrine-resistant cell models described earlier. Interestingly, Inhibition of Src phosphorylation in these cells using the Src kinase inhibitor, saracatinib, restores cell–cell contacts and results in reorganisation of the cells into tightly packed epithelial cell colonies similar to that of their parental, endocrine-sensitive cells (86). Underlying this phenomenon is likely to be a reversal of the Src-dependent increase in beta-catenin phosphorylation since in anti-hormone-resistant cells, catenin phosphorylation is elevated as a consequence of elevated Src activity (25, 27). Moreover, in addition to its role as a mediator of intercellular adhesion, Src is also intimately linked with FAK to regulate cell-matrix attachment and cell migration (see (79) for a review). Indeed FAK, Src and their associated protein, paxillin, have been considered to act together as a functional unit in which all components must be present to achieve optimal cell–matrix adhesion. Changes in Src activity can directly influence FAK activation state and the subsequent migratory capacity of the cell and suggest an additional reason for the observed increase in FAK activity in endocrine-resistant cell models which may be separate from that due to integrin changes.

6. ACQUIRED RESISTANCE TO ENDOCRINE AGENTS PROMOTES AN ANGIOGENIC PHENOTYPE

Adequate vascularisation of the tumour mass is required for delivery of nutrients and oxygen to the growing tumour with hypoxa-induced signalling within the tumour resulting in production of pro-angiogenic factors that promote its subsequent neovascularisation. Angiogenesis also plays a central role as a facilitator of the metastatic process, allowing a point of access for the tumour cells to the host circulatory system. It is thus not surprising that the extent of tumour vascularisation correlates with presence of metastases. Clearly, therefore, events that elicit pro-angiogenic responses form tumour cells may play important roles in promoting tumour dissemination.

We have recently observed that endocrine resistant breast cancer cells show increased expression of a number of pro-angiogenic factors (e.g. VEGF, IL-8) in addition to a reduction in the expression of angiostatic factors (E. Hayes, unpublished observations). Our preliminary studies have shown that human umbilical vein endothelial cell (HUVEC) cultures stimulated by conditioned medium from resistant cells show enhanced proliferation compared with conditioned medium from endocrine-sensitive counterparts and this is accompanied by an elevation in HUVEC ERK1/2 activity. Significantly, conditioned medium from endocrine-sensitive MCF7 cells engineered to express constitutively active Src also stimulate HUVEC proliferation whereas conditioned medium from antioestrogen-resistant cells treated with a Src kinase inhibitor fails to elicit angiogenic activity in HUVEC cultures. This is interesting in the light of recent reports that Src signalling via FAK has been identified as a mechanism for the production of VEGF and subsequent blood vessel growth *in vivo* (87). Notably, pharmacological inhibition of Src can reduce FAK tyrosine phosphorylation in a number of tumour cell types, including our acquired resistant cells (72, 88), suppress VEGF and IL-8 expression (89, 90) and prevent VEGF-induced proliferation of endothelial cells (91).

7. CONCLUSIONS

Evidence is increasing which reveals that prolonged exposure to endocrine agents results in a number of changes within breast cancer cells that favour an adverse, pro-invasive phenotype *in vitro*. Such changes include the overexpression of a number of cell surface receptors which may sensitize these cells to factors found within the tumour microenvironment. Indeed, the concept that the development of endocrine resistance in breast cancer cells sensitizes these cells to stromal-produced factors is further supported by experimental data showing the ability of conditioned medium from primary fibroblast cells to promote the migration of endocrine- resistant breast cancer cells compared to their endocrine-sensitive counterparts although it is not currently clear which fibroblast-secreted factors and/or epithelial cell receptors are involved in this process. However, these observations have clear implications for the development and spread of tumours in an *in vivo* context. Several potential targets for intervention have been identified through which these adverse cellular features may be suppressed; although there are few inhibitors available for c-Met and CD44 is not yet developed as a target, the targeting potential of these individual molecules has been demonstrated through siRNA studies. Src also plays a fundamental role in anti-hormone resistance, where it appears to drive the development of an aggressive phenotype, at least *in vitro*, and in part through its ability to modulate both cell–cell and cell–matrix interactions. Of particular importance is the potential use of pharmacological inhibitors of Src or FAK in breast cancer, whey may represent novel anti-invasive agents to limit tumour progression. Importantly, therapeutically targeting these latter molecules may also have the additional benefit when combined with standard chemotherapies, to achieve greater response and potentially

delay or prevent emergence of an aggressive, resistant phenotype.

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