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Molecular basis of invasion in breast cancer

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Abstract. Cancer cell invasion involves the breaching of tissue barriers by cancer cells, and the subsequent infiltration of these cells throughout the surrounding tissue. In breast cancer, invasion at the molecular level requires the coordinated efforts of numerous processes within the cancer cell and its surroundings. Accumulation of genetic changes which impair the regulation of cell growth and death is generally accepted to initiate cancer. Loss of cell-adhesion molecules, resulting in a loss in tissue architecture, in parallel with matrix remodelling may also confer a motile or migratory advantage to breast cancer cells. The tumour microenvironment may further influence the behaviour of these cancer cells through expression of cytokines, growth factors, and proteases promoting chemotaxis and invasion. This review will attempt to summarise recent work on these fundamental processes influencing or facilitating breast cancer cell invasion. (Part of a Multi-author Review)

Keywords. Breast cancer, invasion, cell adhesion, EMT (epithelial-mesenchymal transition), ECM (extracellular matrix), migration, microenvironment.

Introduction

Breast cancer is by far the most common cancer in women in Europe. The most recent publication from the European Network of Cancer Registries displays some alarming statistics [1]. In 1995 alone, 321 000 new cases of breast cancer were diagnosed, accounting for over one quarter of all female cancers. Breast cancer further accounted for 17 % of all European female cancer deaths, with 124 000 women succumbing to the disease.

In addition to high incidence and mortality rates, few non-hormonal ER/PR (estrogen receptor and progesterone receptor)-targeted therapies exist for breast cancer management and treatment. Trastuzumab (Herceptin), a monoclonal antibody against the growth factor receptor HER2, has been shown to dramatically improve survival in HER2-over-expressing breast cancer patients, alone and as an adjuvant therapy in combination with other treatments [2, 3]. However, few other breast cancer targets have been successfully identified and exploited for the development of new and better drugs.

Breast cancer is a heterogeneous disease, forming within the breast ducts or lobules, and it has been suggested that there are multiple pathways of progression to an invasive cancer. One possible paradigm for the progression of ductal breast cancer, which accounts for 80 % of all breast cancer diagnoses, is from normal pathology through atypical ductal hyperplasia (ADH) to ductal carcinoma *in situ* (DCIS), then invasive ductal carcinoma (IDC), and culminating in metastatic disease [4] (Fig. 1). DCIS can be defined as a proliferation of cancer cells situated within the confines of the breast duct. Ductal breast cancer invasion, therefore, requires that cancer cells breach the epithelial cell basement membrane and migrate out of the duct into the surrounding tissue. Consequently, invasive cancers may spread into surrounding

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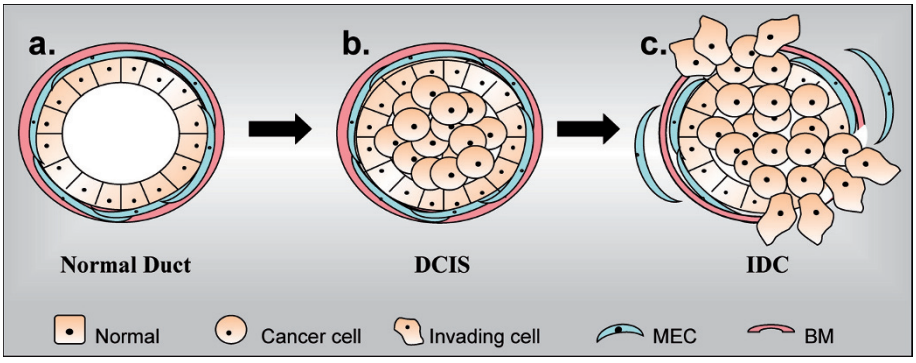

Figure 1. Ductal breast cancer progression. Ductal breast cancer is generally accepted to progress through these main stages. Normal, defined by a thin layer of epithelial cells lining the duct lumen (*a*) Ductal carcinoma *in situ* (DCIS), in which cancerous cells have proliferated to fill the breast duct (*b*) Invasive ductal carcinoma (IDC), at which stage myoepithelial cell (MEC) presence is diminished and ductal cancer cells have breached the epithelial cell basement membrane (BM) and invaded into surrounding stroma (*c*).

tissue, enter local vasculature, and metastasise to distant sites, the occurrence of which often leads to eventual death in many cancer patients. Understanding the mechanisms which facilitate the invasive transition in breast cancer is therefore of the utmost importance. It is likely that numerous factors, both intrinsic to cells and additionally extrinsic environmental cues, play key roles in the pathogenesis of breast cancer invasion.

This review will attempt to summarise recent work concerning alterations within breast cancer cells and those of the tumour microenvironment, and also the impact of cell adhesion, extracellular matrix (ECM) proteins, and cell motility in the migration and invasion of cancer cells.

Gene-expression profiling of breast cancer

Breast cancer arises through abnormal genetic alteration and/or gene expression events. It is a heterogeneous disease, and multiple genetic aberrations have been identified that may have a causal role in initiation and progression. As mentioned, metastasis of primary breast cancer to distant sites is the main cause of cancer fatalities, and substantial efforts have been made to elucidate changes that may predict invasion and subsequent metastasis. To this end, many studies have been conducted on breast cancer progression, invasion, and metastasis, in an effort to identify genes whose altered expression is significantly linked to breast cancer. Genes identified may ultimately prove useful as potential future predictive and prognostic targets, identifying high-risk patients, and preventing over- or undertreatment of breast cancer patients. Genes may also be possible drug targets, allowing development of new and better targeted therapies for breast cancer patients, ultimately improving overall and recurrence-free survival rates.

This section of the review will focus on recent work utilizing microarray technology. To date, this work has produced several interesting breast cancer target gene candidates, shed light on invasive and metastatic gene signatures, and improved the sub-grouping of breast cancer patients.

Breast cancer subtypes and prognosis

The advent of microarray technology has revolutionised the field of research genetics. Microarrays have enabled high-throughput whole-genome gene expression profiling in unprecedented time limits. This wealth of information has been further utilised and explored via a range of new and sophisticated software suites allowing normalisation and comparisons of expression profiles across multiple arrays and multiple platforms. These advances have enabled researchers to uncover valuable data regarding many diseases, including the heterogeneity of genetic changes in breast cancer progression.

A key finding utilising this technology has allowed further important refinements in the classification of breast cancer subtypes via hierarchical clustering of multiple gene expression profiles of human breast tumours. Four distinct breast cancer subtypes or 'portraits' were identified, each differing in biological characteristics and clinical outcome: normal breast-like, luminal-like, ERBB2-positive, and basal-like [5]. Broadly divided based on ER status, the normal-like ER-positive subtype is characterised by high expression of genes of basal epithelial cells and adipose tissue, while tumours expressing ER are characterised by expression of many genes expressed by breast luminal cells. ERBB2-positive tumours overexpress the ERBB2 oncogene but show low levels of ER and ER-regulated genes; basal-like tumours are largely

ER-negative and express breast basal cell keratins [5]. Differences in the prognosis of tumour subtypes have been shown, with luminal-like and basal-like tumours associated with good and poor prognosis, respectively. ER-negative basal-like and ERRB2 subtypes are further associated with strongly reduced overall survival [6]. Additionally, these molecular subtypes reflect the inherent properties of tumours, and seem to be further sustained throughout chemotherapy treatment as well as between primary tumours and their lymph node metastases [7].

Ultimately, gene expression profiling of primary breast tumours could predict clinical outcome, and prognostic gene signatures may allow early identification of patients at high- and low-risk of aggressive cancer. Sotiriou et al. assessed the gene expression profiles of breast cancer patient datasets and identified a gene expression index associated with cancer grade. This index allowed refined classification of many grade II patients into a further high and low risk grouping. This approach may, in future, improve the accuracy of tumor grading and thus its prognostic value [8]. In a seminal study by Van't Veer et al., the genetic profiles of breast tumours with and without distant metastasis at 5 years identified a poor prognosis signature. This 70-gene signature, identified using a three-step supervised clustering method, includes genes involved in cell cycle, angiogenesis, invasion and metastasis, and signal transduction [9]. This signature has been shown to be a powerful indicator of distant metastasis and a more powerful predictor of disease outcome in young patients than standard systems based on clinical and histologic criteria alone [10, 11]. In the future, the genetic profiling of patients may lead to a more individualised tailored adjuvant treatment, which could greatly reduce adverse side effects and healthcare expenditure by reducing over- or undertreatment of patients.

Breast cancer metastasis

As poor prognosis is inextricably linked with metastasis, gene expression profiling has additionally been exploited to attempt to determine metastasis gene signatures to particular preferential distant sites. Mouse models of metastatic breast cancer have been invaluable in identifying genes that may be key factors in the metastatic process of human cancers. For example, a set of genes that mediate breast cancer metastasis to lung was identified by injection of MDA-MB-231 human breast cancer cells into immunodeficient mice. Isolated lung metastatic cells were harvested and re-injected to yield a highly metastatic derivative cell line. Subsequently, a lung metastasis signature of 54 genes, examples of which include MMP1, SPARC, and VCAM1, was identified by comparing gene expression profiles of parental and derivative cell lines [12]. In a parallel study, MDA-MB-231 cells were similarly used to identify a bone metastasis gene signature. No enhancement of the previously identified poor prognosis 70-gene set was observed, indicating the involvement of a new set of bone metastasis genes, including cell membrane and secretory products that may affect the host environment, thereby favouring metastasis [13]. A further alternative set of bone metastasis-related genes was identified by inoculation of mice with a bone-metastatic mouse cell line [14]. A high proportion of the genes identified were ECM molecules, again implicating the importance of the host environment in promoting metastasis. Functional assays in both lung and bone metastasis mouse models show that overexpression of a combination of metastasis-related genes leads to more aggressive metastatic activity *in vivo*. These studies have highlighted a number of genes that may have an important function in the pathogenesis of human breast cancer, and also suggest that the coordinated effort of a number of genes is required to develop metastasis.

Gene expression profiling of human primary and metastatic tumours has also enabled identification of metastasis-related genes. This gene signature distinguishes human primary tumours from metastatic adenocarcinomas and has shown that a subset of primary tumours closely resemble metastatic tumours [15]. Solid tumours carrying the metastasis signature are associated with metastasis and poor outcome, again suggesting that the metastatic potential of tumours may be encoded in the primary tumour. This, and the observation that many metastasis-related genes are involved in ECM interactions with the host environment, supports the 'seed-and-soil' hypothesis of metastasis. This hypothesis suggests that metastasis depends on crosstalk between the cancer cells (the seeds) and the host microenvironment (the soil), and that both the potential for metastasis and the correct environment is required for metastasis to occur [16].

Breast cancer invasion

Metastatic gene signatures may ultimately be important in the clinical management of breast cancer. However, metastasis of ductal breast cancer first requires the transition from an *in situ* breast tumour to an invasive tumour. Many researchers have attempted to determine which genetic alterations, differing between these two disease states, may be applicable as therapeutic targets for the prevention of invasion and therefore subsequent metastasis. Progressive stages of human breast cancer have been compared

using gene expression analysis to attempt to identify key invasion-promoting genes [17–21].

Ma et al. used Laser Capture Microdissection (LCM) to collect pure populations of normal breast cells, DCIS, and IDC cells and conducted genetic profiling using cDNA microarrays. Twenty-nine genes were found to be overexpressed in IDC relative to matched DCIS specimens. However, gene expression differences between distinct pathological stages of breast cancer were not identified [18]. In a similar gene expression study, profiles of patient-matched DCIS and IDC samples were shown to genetically resemble their matched counterparts more closely than tumours of a similar grade, perhaps reflecting the heterogeneity of breast cancer [21]. A master set of genes differing significantly between DCIS and IDC was identified, including genes such as BPAG1, MMP11, and GREM1, that may have clinical significance. It should be noted, however, that only four genes, adipocyte enhancer-binding protein 1 (*AEBP1*), syndecan 2 (*SDC2*), chromosome 18 open reading frame 1 (*C18orf1*), and collagen type XV A1 (*COL15A1*), were found to be in common between the two above similar studies. It seems apparent that no definitive DCIS or IDC gene signature, and therefore no definitive invasive signature, has been identified to date. In a different approach to solving this problem, other researchers have exploited one of the known properties correlating with metastasis: chemotaxis to blood vessels. Using an *in vivo* invasion assay Wang et al. identified a gene signature of invading cancer cells. This assay collected invading cells from rat mammary primary tumours via a needle containing Matrigel and EGF (epidermal growth factor). Invading cancer epithelial cells were then compared to cancer cells from the bulk of the mammary tumours [22]. The most dramatically changed genes within this invasion-associated gene signature were those involved in cell division, survival, and cell motility. This indicates that the motility machinery of cancer cells is extremely important in invasion. Furthermore, ZBP-1, a gene that restricts the localisation of a major motility machine component, β-actin, was further shown to be dramatically down-regulated in invasive cells, suggesting that ZBP-1 is a master gene regulating invasion and metastasis.

In summary, gene expression profiling has been successful in identifying genetic signatures indicative of diagnosis and prognosis, including invasion and metastasis. However, little overlap has been seen between these gene expression studies. Additional work with invading cells collected from human tumours will be required to further elucidate the changes involved in invasive transitions. These studies will hopefully identify genes suitable for therapeutic targeting, which may eventually allow the prevention of invasion and metastasis, the most alarming aspect of breast cancer.

Cancer cell motility and migration

As outlined, the genetic background to breast cancer invasion is complex and multi-factorial. Although targets controlling tumour cell proliferation and apoptosis have attracted much attention in recent years, it must be remembered that additional functions such as the acquisition of a motile phenotype are essential for tumour cells to become invasive. This has been highlighted by recent studies reporting that invasive cells at the margins of mouse tumours exhibit genetic profiles that are non-proliferative and non-apoptotic relative to the main tumour, but instead feature significant upregulation in genes controlling cell motility [22]. Thus, this section of the review will concentrate on the contribution of cell migration to breast cancer invasion, particularly the migratory mechanisms used by tumour cells to force their way out of a primary carcinoma into a secondary site. It will also discuss the concept of 'migratory plasticity', or how tumour cells can alter their migratory phenotype to remain motile under changing micro-environmental conditions. Finally, it will touch upon some therapeutic considerations relating to the use of anti-motility agents in the treatment or prevention of invasive breast cancer.

Basic principles of cell migration

Cell migration is fundamentally important for a myriad of physiological functions in all living creatures. High levels of motility associated with simple amoeboid organisms allow them to efficiently seek out food sources, while more complex organisms possess a range of cell types whose migration is tightly regulated according to physiological need. During vertebrate development, for example, cell migration is essential for organogenesis and the generation of polarity. In developed organisms, cell motility plays key roles in neuronal communication and the trafficking of leukocytes. However, cell migration is also of critical importance in pathophysiological settings such as wound closure and cancer. In the context of breast carcinoma, the ability of epithelial cells at the edge of a histologically confined tumour to migrate away from the primary site is an early determinant of the transition from an *in situ* into an invasive phenotype. Furthermore, since metastasis cannot happen without initial invasion [16], a better understanding of the migratory mechanisms used by cells is important for our understanding of some key events influencing mortality in breast cancer.

Mechanisms of cell migration

Cell migration can be broadly divided into two forms, encompassing single-cell migration (performed by leukocytes and fibroblasts) and coordinated cell migration (also called collective or cohesive), which is observed at barrier surfaces of the body, including epithelia and endothelia. The key difference between these two forms is that single cells can move independently, whereas cells at barrier surfaces are firmly attached to each other via intercellular junctions and therefore must 'crawl' forward as sheets or chains in which their neighbours remain attached [23]. This important difference notwithstanding, both single cell and coordinated cell migration involve dynamic and cyclical sequences of events which can be summarised as follows [24, 25]. The first step involves extension of a protrusion in the desired direction of movement. Such protrusions include thin, hairlike filopodia and broad veil-like cytoplasmic protrusions known as lamellipodia, which are induced upon activation of Rho family small GTPases Cdc42 and Rac1, respectively [26–28]. The second step requires stabilisation of this interaction through the assembly of transient focal contacts with the ECM, formed via integrin clustering in the cell membrane with subsequent recruitment of linker proteins like paxillin forming a bridge between the cell membrane and the F-actin stress fibre network. The third step involves contraction of this network mediated by interactions between F-actin and the newly phosphorylated light chain of myosin II, in a process generating sufficient tension to drag the cell forward. The fourth and final step requires disassembly of F-actin-linked focal complexes at the rear of the cell, loosening the grip of the migrating cell on the matrix and allowing it to retract and be dragged in the direction of migration. The cycle will then begin again, resulting in a continuous forward drive as long as the microenvironmental conditions favour migration.

While this paradigm evolved to explain cell migration on flat surfaces in two-dimensional systems, with the addition of one step it is now accepted to occur also within more complex three-dimensional environments (including ECM *in vivo*). This additional step involves recruitment of matrix-degrading enzymes such as matrix metalloproteinases (MMPs) to sites where integrin complexes have clustered, allowing localised proteolysis of the ECM and downward extension of cellular projections known as invadopodia [29]. Adding to the four-step paradigm described above, this event would occur after the formation of focal complexes and before actomyosin contractility events which generate the tension necessary to drag the cell body forward. The role of matrix-degrading enzymes in cancer will be dealt with in a subsequent section of this review; this section will focus more on the physical considerations associated with migration. This is not to discount the importance of matrix remodelling in cancer cell migration and invasion, but merely to note the gaps in our understanding of the spatial and temporal contributions of MMPs to the invasive process. The disappointing performances of MMP inhibitors in *in vivo* clinical trials of invasive breast cancer [30] have served to further emphasise this point. However, recent investigations have shed light on possible explanations for this phenomenon, namely that matrix degradation does not only facilitate cancer cell migration but also liberates potent anti-angiogenic substances [31]. Therefore, the transient benefit of matrix degradation in facilitating cancer cell migration may be superseded at a higher level by inhibitory effects on the survival of secondary tumours.

Tumour cell migration – single cell and coordinated migration

It is thought that tumour cells migrate similarly to normal cells; however, several factors could make migration either more efficient or more sustained. While pro- and anti-migratory signals are strictly balanced in normal cells, much evidence suggests that tumours have a preponderance of pro-migratory signals, such as high expression levels of growth factor receptors. Overexpression of the EGF receptor family member Her2 in breast carcinomas [32], which in turn spurred the clinical development of the Her2 antagonist Herceptin [33], is perhaps the best 'bench-to-bedside' illustration of this concept. However, growth factor receptor over-expression is by no means a phenomenon confined to breast tumours [34]. Other mechanisms that could tip the balance in favour of migration include elaboration of growth factors (e.g. EGF) or cytokines (e.g. hepatocyte growth factor/scatter factor, HGF/SF) by breast tumours [35]. As a representative example, HGF/SF facilitates migration by activating actin-restructuring enzymes such as Rho kinase and PAK [36], or by enhancing turnover of migratory machinery at spreading edges [37].

A key consideration in breast cancer invasion is whether tumour cells migrate singly or coordinately. Since breast carcinomas form at epithelial sites, at which cells are joined to their neighbours by intercellular junctions, we might therefore expect coordinated cell migration to be the predominant migratory phenotype. This indeed appears to be the case in well-differentiated lobular carcinomas of the breast [38]. However, structural and functional abnormalities in intercellular adhesion proteins reported in breast cancer (as discussed later in this review), which correlate negatively with differentiation status, may

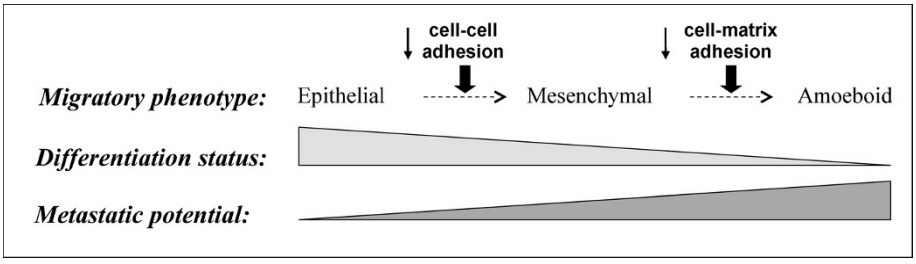

Figure 2. Putative role of adhesion in the relationship between migratory phenotype, differentiation status and metastatic potential. Breast tumours with a polarised epithelial phenotype, including intact cell-cell and cell-matrix junctions, are considered to be well-differentiated and although potentially invasive are unlikely to metastasise. Loss of this epithelial phenotype, such as that resulting from disassembly of cell-cell junctions, correlates with de-differentiation, mesenchymal-like migration and increased risk of invasion/metastasis. Subsequent loss of cell-matrix adhesive junctions would facilitate additional de-differentiation from a mesenchymal to an amoeboid mode of migration, potentially increasing metastatic potential.

signal a switch from coordinated migration to single-cell migration.

The possibility that epithelial cells within a carcinoma could begin to migrate independently from each other has important functional implications for breast cancer invasion. In theory, single cells have the potential to move through smaller spaces and to travel longer distances than cells constrained by the necessity to move as sheets or chains. Nonetheless, tumour epithelial cells which migrate as a unit can still be locally invasive [38]. However, since these are likely to belong to well-differentiated tumours, it could be argued that tumour epithelial cells which migrate collectively pose a smaller risk of forming metastases than do individually migrating cells from poorly differentiated tumours.

Escape mechanisms used for tumour cell migration

Regarding the actual migratory mechanisms used by single epithelial cells, this too can have a significant impact on invasion status. Single cells can migrate through one of two predominant mechanisms. The first is termed mesenchymal movement, whereby cells possess an elongated fibroblast-like shape and move rapidly through channels cut into the surrounding matrix by matrix-degrading enzymes such as MMPs [39]. This mechanism has been shown to be involved in the invasion of breast cancer cells and many others. However, it has been observed that blocking MMP activity is not sufficient to abrogate cancer cell invasion [40], which has opened up much discussion on the second mode of single-cell migration.

The second type of single-cell migration is a protease-independent form known as amoeboid movement, so named because cells resemble primitive unicellular organisms which rely on shape deformations to move through small, inflexible pores in their surrounding matrix. This form of migration engages signalling pathways such as Rho/Rho kinase, generating mechanical force allowing cells to squeeze their way through the matrix without the need for protease-cleared tracks [39]. Although it is believed that invasive tumour cells mostly use the mesenchymal motility pathway, recent work has shown that tumour cells are capable of 'switching' from a mesenchymal to an amoeboid mode under conditions when pericellular proteolysis is blocked [41] or when Rho/ROCK activity is at high levels [42].

This transition, named mesenchymal-to-amoeboid transition (MAT), has profound implications for both the aetiology and treatment of invasive breast cancer. If cancer cells *in vivo* can indeed switch between modes of motility, it may offer them an 'escape strategy' to circumvent a potential shutdown of their migratory activity. Such pleiotropism is likely to render them more adept at forming secondary tumours, and in addition more difficult to treat. The postulated link between migratory mode, differentiation status and MAT is summarized in (Fig. 2).

Prospects for anti-migratory cancer therapy

Much recent interest has centred on the possible clinical uses of anti-motility agents to arrest cancer cell invasion out of primary tumours [43]. This is a particularly valid strategy if invasive cells do indeed possess a phenotype which is preferentially pro-migratory rather than pro-proliferative or anti-apoptotic [44], as this might render them insensitive to current chemotherapeutic agents. However, an important consideration is that systemic application of anti-motility agents would have the potential to collapse normal physiological functions including (but not limited to) immune cell trafficking and neuronal communication. Intratumoural administration of anti-motility agents would thus be preferable, although in turn this strategy might be of limited value unless used at an early stage before tumour invasion. A big advantage, however, would be that targeting the machinery common to all motile cells would block the escape route of cells switching their motile phenotypes, whether from coordinated motility to individual motility

or indeed from mesenchymal to amoeboid motility. It is clear that much remains to be explored before the advent of anti-motility agents either as stand-alone or adjunct therapies for invasive breast cancer.

The impact of the tumour microenvironment

Although the migration of cancer cells is a prerequisite for invasion and metastasis, the host environment plays a major role in facilitating both these processes. Cytokines, growth factors, and proteases secreted from multiple cell types into the tumour microenvironment may have a profound effect on potential invasion and later metastasis of breast cancer primary tumours. This section will discuss the increasing evidence emerging to support a role for cells in the tumour microenvironment in initiating tumourigenesis. For the purposes of this review we will focus on the main cell types of the tumour microenvironment – fibroblasts, myoepithelial cells and macrophages – and discuss how these cells may ultimately prove to be key players in initiating and driving both human breast tumour formation and invasion.

Fibroblasts

Normal fibroblasts have important functions in the deposition of the ECM and formation of the basement membrane, via the synthesis of collagens, fibronectin, and laminin [45]. They are also crucial regulators of inflammation, epithelial differentiation, and wound repair. In normal wound healing, fibroblasts invade wounds and become 'activated', generating large amounts of ECM proteins, which aid in the healing process. After healing, the number of activated fibroblasts dramatically decreases. However, in the tumour environment fibroblasts remain permanently activated, proliferating at a faster rate and also secreting higher levels of ECM proteins than fibroblasts in healthy tissue [45,46]. Indeed, in many breast cancers, the 'hardness' of a breast lump is largely attributable to the high proportion of collagen and ECM surrounding the tumour [47]. Fibroblasts associated with malignancy, generally referred to as carcinoma-associated fibroblasts (CAFs), represent the most abundant cell type in the tumour stroma [48]. These observed similarities between tumour stroma generation and wound healing led to tumours being described as 'wounds that do not heal' [47]. Seminal work supporting this hypothesis and indicating that active stroma may have a profound effect on tumour formation came from work on cancer-susceptible chickens. Wounding of these chickens, infected with the Rous sarcoma virus (RSV), led to tumour formation with nearly 100 % frequency in wounded tissues that would otherwise remain tumour-free if no wound was present [49].

Gene-expression profiling of cells present in the tumour microenvironment has also uncovered some interesting results. Gene expression changes have been reported to occur in all cell types during cancer progression [50]. Of note was the high expression in CAFs (carcinoma-associated fibroblasts) of a chemokine, CXCL12, which binds to receptors on epithelial cells, enhancing their proliferation, migration, and invasion. This indicates that fibroblast chemokines may play a role in breast tumorigenesis by acting as paracrine factors [50]. Cell culture experiments have further shown the influence of fibroblasts on tumour cell invasion and migration *in vitro*, as conditioned medium collected from fibroblasts has been shown to stimulate both the motility and invasion of numerous breast cancer cell lines [51].

Mouse models have lent further support to the importance of the tumour microenvironment, including fibroblasts, in promoting cancer inititation and growth. Irradiation of mammary gland stroma promotes tumorigenesis in unirradiated epithelial cells in mouse mammary models [52]. More recently, Kuperwasser et al. utilised a xenograft mouse model in which both the stromal and epithelial components of the reconstructed mammary gland were of human origin. Transplanted human fibroblasts, expressing HGF (hepatocyte growth factor) or TGF-β (transforming growth factor beta) together or alone, initiated DCIS and invasive breast carcinomas, whereas transplantation of normal fibroblasts did not [53]. In a similar study, human CAFs or normal human fibroblasts were mixed with MCF-7-ras human breast cancer cells and inoculated into immunodeficient nude mice [54]. CAFs were more competent than their normal counterparts in enhancing breast tumour growth, giving rise to highly vascularised tumours. Additionally, CAFs produced increased levels of stromal cell-derived factor (SDF-1), responsible for boosting tumour angiogenesis and enhancing tumour growth by direct paracrine stimulation via CXCR4 receptors on human breast carcinoma cells. These observations and results provide compelling evidence of the impact of fibroblasts on tumour development and progression.

Myoepithelial cells

In contrast to the tumour-promoting properties of fibroblasts, myoepithelial cells (MECs), which localise in the normal breast between luminal epithelial cells and the stroma, have been described as the tumour suppressor cells of the mammary gland [55, 56]. They play a major role in breast development by inducing cell polarity and ductal morphogenesis, as well as in

lactation and the formation of the basement membrane due to their expression of oxytocin receptors, smooth muscle actin, collagens, and laminin. [57, 58]. MECs have been suggested to be natural tumour suppressors due to their ability to inhibit breast cancer cell growth, invasion, and angiogenesis [55, 59, 60]. Unsurprisingly, the loss of normal MEC function and therefore normal breast function is almost universally associated with breast cancer [57]. In ductal breast cancer, MECs generally continue to surround DCIS lesions but are absent in many invasive breast cancers [55]. In fact, the degradation of both the MEC layer and the basement membrane is an absolute prerequisite for breast cancer invasion and metastasis [61], suggesting a possible gatekeeper role of MECs in breast cancer invasion. Researchers have therefore recently focussed on the differences in the behaviour of normal and cancer-associated MECs. It has been shown that cancer-associated MECs are unable to induce correct luminal cell polarity, the loss of which is a hallmark of cancer progression, possibly due to an inability to produce laminin-1 [58].

Immunohistochemical analysis of normal breast and DCIS has identified differentially expressed proteins between normal and cancer-associated MECs, which may be useful as markers of tumour progression [62, 63]. For example, MEC expression of lysyl oxidase, an enzyme important in ECM stability, was found to be decreased in DCIS-associated MECs compared to normal MECs [62]. Furthermore, serial analysis of gene expression (SAGE) profiles from purified populations of all cell types in normal breast and breast tumour microenvironments has revealed that the majority of gene expression changes occurred in myoepithelial cells [50]. A large fraction of these genes were reported to encode secreted or cell surface proteins, suggesting extensive abnormal paracrine interactions between myoepithelial and other cell types. Additionally, alterations in the DNA methylation patterns of normal and cancer-associated MECs has also been reported, which may indicate a major impact on the gene expression changes in cancer MECs. [64]. These data suggest an important role of cancer-associated MECs in breast tumour progression, with one hypothesis suggesting a 'release' model of breast cancer invasion in which phenotypic changes in myoepithelial cells, in coordination with the infiltration and influence of inflammatory cells, lead to the breakdown of the ducts and the release and invasion of tumour epithelial cells [56].

Macrophages

Another highly represented cell in the breast tumour microenvironment is the macrophage. Macrophages function normally in wound healing and infection by promoting tissue repair and initiating immune responses via cytokine and chemokine interactions with other cells [65]. They have also been shown to play a role in development and morphogenesis of many tissues. For example, mice deficient in macrophages show, among other defects, aberrant morphogenesis of the mammary gland [66, 67]. In cancer, the tumour microenvironment is often populated by haematopoietic cells, including macrophages, which have been shown to facilitate angiogenesis and ECM breakdown and to promote tumour cell invasion and migration [68, 69]. Tumour-associated macrophages (TAMs) have been shown to influence angiogenesis via factors such as vascular endothelial growth factor (VEGF), angiogenin (ANG)1 and ANG2 [67, 69, 70]. TAM accumulation is associated with expression of macrophage chemoattractants, and both correlate significantly with levels of potent angiogenic factors such as VEGF, thymidine phosphorylase (TP), tumour necrosis factor-alpha (TNF-α), and interleukin-8 (IL-8) [71]. Inflammation has long been associated with cancer initiation, and recruited TAMs constitute a substantial portion of the tumour mass [72]. In fact, high numbers of TAMs are associated with poor prognosis in breast cancer [68]. It has been suggested that the cytokine profile in the tumour microenvironment can block maturation of antigen-presenting cells and influence macrophages to be trophic for tumour cells, thus promoting tumour progression and invasion [67,73]. Illustrating this point, overexpression of a major regulator of macrophage recruitment and activity, colony stimulating factor 1 (CSF-1), has been correlated with poor prognosis of human breast cancers [73]. Furthermore, when CSF-1-null mutant mice are crossed with breast cancer-susceptible PyMT mice, tumours occur in both wild-type and CSF-1-null mice. However, the absence of CSF-1 results in a marked delay in tumour progression and metastasis [73]. A possible mechanism for this CSF-1-induced invasion was investigated through *in vivo* and *in vitro* studies of tumour cell invasion [74, 75]. It was shown that both tumour cells and macrophages co-migrate and depend on each other for invasive potential. A CSF-1/EGF paracrine loop is generated whereby CSF-1 expression by tumour cells promotes the expression of EGF by TAMs. EGF, in turn, further promotes the growth and invasion of tumour cells [74]. Disruption of this loop was shown to be sufficient to inhibit tumour cell and macrophage invasion and migration. These results indicate that macrophages can contribute to cancer initiation and progression by adopting a trophic role in the tumour microenvironment, encouraging cancer growth and invasion.

The studies reviewed provide compelling evidence that several cells in the tumour microenvironment can

directly influence cancer growth and invasion. Intrinsic changes may confer invasive capacity to cancer cells, but extrinsic factors from the microenvironment may also be required to initiate tumour cell invasion *in vivo*, as discussed in the next section of this review [76].

The extracellular matrix in cancer invasion

Another important factor in the tumour microenvironment is the ECM. Many important matrix-remodelling proteins present in the ECM, such as proteinases, have long been implicated in breast cancer invasive transitions. This review section will discuss the major enzymes implicated in ECM remodelling, thereby facilitating the invasion of cancer cells.

The ECM is a complex and dynamic meshwork that provides structural support in the form of bone, cartilage, and tendon. The ECM also plays an important role in many biological processes, such as cell adhesion, migration, and invasion, as well as cell proliferation and differentiation. Degradation and remodelling of the ECM can have profound effects on both biological and pathological processes. Various types of proteinases are implicated in ECM degradation, but the major enzymes in this process are considered to be the MMPs [77].

The extracellular matrix

The ECM can generally be divided into two categories: the basement membrane and the interstitial connective tissue [78]. The basement membranes are extracellular structures that usually separate cells from the underlying connective tissue. The major components of the basement membranes are type IV and V collagen, laminin, entactin, and several glycoproteins, which interact non-covalently to form a dense network. In the adult, the basement membrane usually forms a barrier to most cell types and thus serves to compartmentalise tissues and organs. However, in some cases the basement membrane can be selectively penetrated, for example, during white blood cell diapedesis into surrounding tissue [79].

Included among the interstitial connective tissues are heterogeneous regions such as the dermis and stroma, as well as more specialised tissues such as bone and cartilage. These tissues contain several cell types in addition to those responsible for producing the bulk of the ECM, i.e. fibroblasts in connective tissue and osteoblasts in bone. These additional cell types include macrophages, lymphocytes, melanocytes, endothelial, muscle, and nerve cells. The major protein of the connective tissue matrix is collagen. Collagens type I and III are found in most connective tissues, while types II and IX collagens are present in cartilage. Collagens type VI, VII, VIII, IX, X, and XII have also been described in specific tissues. In addition to the collagens, interstitial connective tissue also contains fibronectin, elastin, chondroitin sulphate proteoglycans, heparin sulphate proteoglycans, tenascin, and hyaluronic acid. Laminin and entactin are also found in interstitial connective tissues and are not exclusively basement membrane components.

The ECM plays an important role in cell invasion, and this is primarily achieved through the interaction of the tumour cell with the ECM via the use of cell adhesion molecules (CAMs). When considering the possible ways in which defects in the ECM may be generated, it is important to remember that the quality and quantity of the matrix depends not only on the structural components, such as collagen, laminin, and proteoglycan, but also on the regulated expression of ECM-degrading proteinases and their inhibitors. The degradation of ECM proteins can be effected by a variety of enzymatic activities, of which there are four main classes [80]: i) serine proteinases e.g. plasminogen activators; ii) cysteine proteinases e.g. cathepsins B; iii) aspartyl proteinases e.g. cathepsin D and iv) MMPs e.g. matrilysin (MMP-7). This section will focus on the latter, MMPs.

Role of MMPs in invasion and metastasis

The MMPs are a family of highly conserved zinc-dependent endopeptidases, which collectively are capable of degrading the components of the basement membrane and interstitial ECM. There are currently at least 24 well-defined members of this family (for a recent review on MMP structure and function, see Nagase et al., 2006 [81]). Degradation of the ECM by MMPs is essential for almost every step of metastasis, for example in aiding cells crossing ECM barriers and the epithelial basement membrane as well as facilitating intravasation and extravasation and colonisation at distant sites [82].

Many studies have investigated the links between tumour invasion and metastasis and MMP expression, and in general it has been concluded that MMPs are inextricably linked to this process [83]. In some cancers, the level of MMP expression can be used as a prognostic marker for how aggressive a particular tumour is. Matrilysin (MMP-7) has been shown to be involved in the invasion and metastasis of several tumours, in particular those of colon and breast origin. Matrilysin has also been shown to play an important role in early breast and colon tumorigenesis [84]. MMP-2 has been found to play an important role in breast cancer and has been detected very early in breast carcinoma but not in normal, resting breast tissue [85]. MMP-2 protein has been localised in tumour cells [86, 87] and also stromal cells [88] in

breast carcinoma by immunohistochemistry. In breast and colon cancer, MMP-9 expression has been correlated with both increased and decreased survival and formation of distant metastasis [89]. In addition, gene expression analysis of human tumors has linked MMP-9 with a poor prognosis in breast cancer [9]. Stromelysin-3 has also been shown to be expressed in breast carcinomas [90], and it was found that stromelysin-3 messenger RNA (mRNA) expression was restricted to the stromal cells immediately surrounding the islands of the malignant tumour epithelial cells. Recent evidence suggests that MMPs play a much broader role in metastasis than previously believed, and that the action of MMPs at steps both before and after the breakdown of the apparent physical barriers to metastasis may in fact be of greater importance [82, 91]. MMPs and their inhibitors appear to be important regulators in the growth of tumours, both at the primary site and at metastases. How MMPs mediate this growth regulation is not yet fully understood, but a number of mechanisms are possible. MMPs have been shown to regulate access to growth factors, such as EGF [92], from the ECM surrounding the growing tumour, either directly or via a proteolytic cascade. Insulin-like growth factor binding proteins (IGFBPs) are cleaved by a number of serine proteinases that are initially activated by MMPs, such as MMP-2 and MMP-9 [93]. Similarly, MMPs and their inhibitors appear to regulate the sustained growth of tumours. Beyond the maintenance of an appropriate growth environment, the role of MMPs in angiogenesis is likely to be important at this stage. Angiogenesis is required for growth of both primary and metastatic tumours, and MMPs play a contributory role in regulating angiogenesis.

Exposure of cells to different matrix components can elicit very different effects [94], and subtle structural changes in the ECM induced by local proteolysis can also influence cell behaviour [95]. Cleavage of ECM components by MMPs has been shown to generate fragments with new functions: cleavage of laminin-5 and collagen type IV by MMP-2 and MMP-14 resulted in exposure of 'cryptic sites' that promoted cell migration [95, 96]. Integrins can also affect the transcription of MMP genes; in an osteogenic cell line, over-expression of $\alpha 2\beta 1$ is associated with an increase in MMP-1 transcription. This could be a way for integrin to act as a signal transducer, influencing the production of MMPs in response to information about the ECM [97].

MMPs as therapeutic targets

As the role of MMPs in tumour development and progression became apparent, many potential inhibitors of these enzymes [metalloproteinase inhibitors (MPIs)] were designed and assessed for anticancer properties. The key to the design of effective MPIs is in understanding the roles and properties of individual MMPs. In this way, indiscriminate effects of such therapies on normal healthy tissue and subsequent side effects can be minimised. Originally, MPI designs were based on small peptides that mimicked MMP substrates [98]. Based on the structure of collagen, the broad spectrum MPI Marimastat (British Biotech, Oxford, UK) showed a significant increase in survival when administered to patients with early-stage, metastasis-free disease [99]. However, Marimastat was not without side effects, and patients experienced extreme joint pains. Trials on BAY 12–9566, an MPI developed by Bayer Pharmaceuticals, were halted because the placebo performed better [100]. To date, Periostat (doxycycline hydrate, a tetracycline analogue) is the only MPI licensed in the United States, and the application is for periodontal disease [101]. The fate of MPI as a therapy lies in the hands of basic researchers. Contributions of MMPs (especially MMP-7) to early stage tumourigenesis suggest it may be beneficial to treat tumours at the early stages using MPIs. Further understanding of the exact roles of specific MMPs at different stages of tumour progression is needed. This would lead to more targeted therapies and should minimise side effects. It is apparent that further research in this area may be fruitful, in terms of our understanding of breast cancer invasion, and also in uncovering and exploiting some valuable therapeutic targets.

Cell adhesion in cancer invasion

As discussed above, remodelling of the ECM is a fundamental process involved in cancer invasion. However, in addition to this, alterations in cell-matrix and cell-cell adhesion may play an important role in breast cancer tumourigenesis. Thus, the final section of this review will discuss this topic.

In general, cell adhesions aid in the organisation and function of epithelial tissues, including the breast, (Fig. 3. Cell-matrix adhesion proteins facilitate the anchoring and interaction of cells with the ECM, whereas cell-cell adhesion complexes allow cells to adhere to each other and to communicate through signals. Together, these help in the formation and maintenance of epithelial tissue architecture. The localisation and expression levels of cell adhesion proteins are fundamental in influencing the polarity of cells and their physiologic functions. However, in breast cancer, alterations of these proteins have frequently been described, and this will be discussed in this review section.

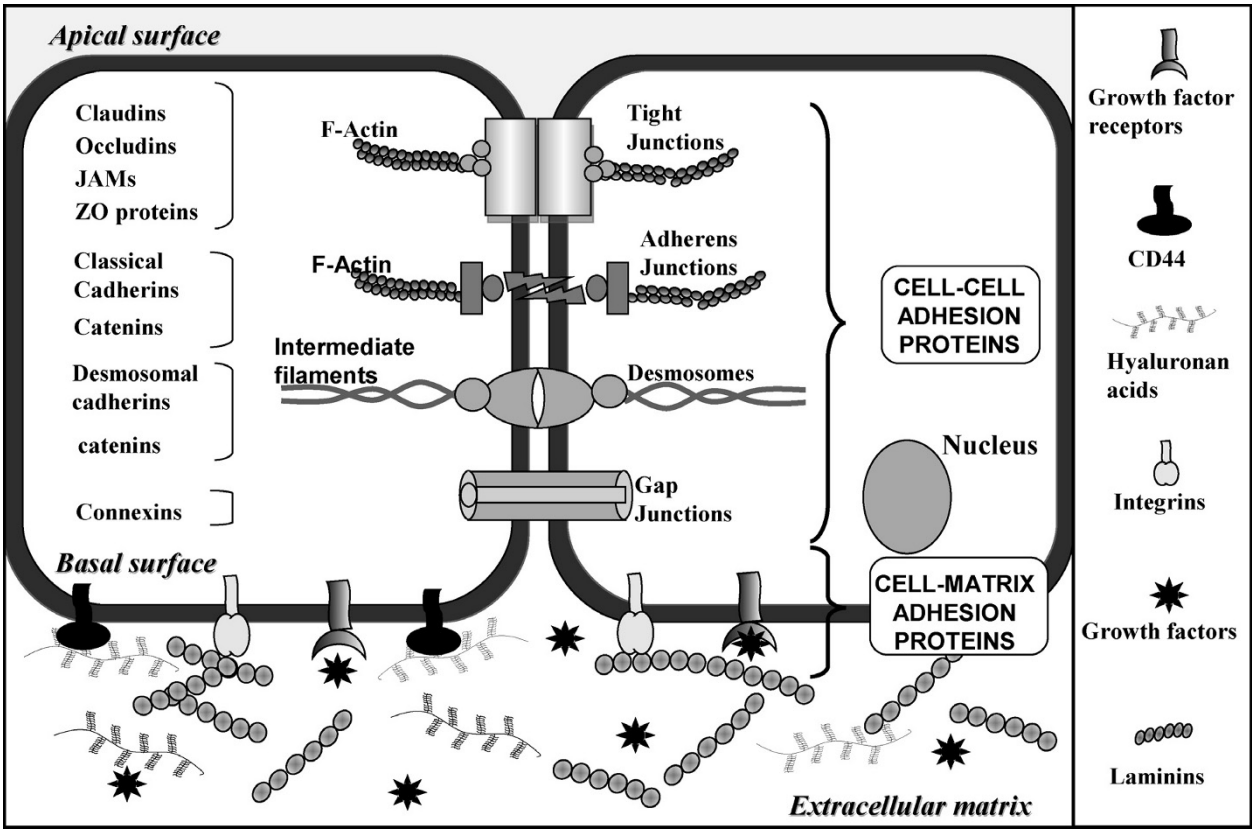

Figure 3. Major molecules involved in cell-cell and cell-matrix adhesion in epithelial cells. Major cell surface receptors and ECM molecules involves in the cell adhesion process are defined in the legend.

Cell-matrix adhesions

ECM receptors function to mediate anchorage of breast epithelial cells to the ECM and to regulate signalling pathways that control actin dynamics, cell movement, growth, and survival. Studies of cell-ECM interactions have been conducted on a wide range of proteins; thus, in this review we will focus only on integrins and CD44.

Integrins are a family of transmembrane glycoproteins that act as receptors for ECM components. In addition to their role in cell migration and adhesion, they participate in events regulating cell-cycle progression and apoptosis through so-called 'outside-in' signalling. They are also involved in 'inside-out' signalling, in which the activation of major cell transduction cascades can influence their activation status [102]. Although there are several integrins involved in cancer progression, the α6 integrins are most highly implicated in breast cancer pathogenesis. In particular, it has been observed that high levels of α6 integrins in breast cancer patients correlate significantly with reduced survival [103]. It has also been described that α6β4 integrin promotes invasion and metastasis by interacting with, and regulating the expression of, the growth factor receptor ErbB2, with subsequent phosphorylation of EGFR and Ras [104]. *In vivo* experiments have shown α6β4 integrin to be implicated in tumour formation and invasion by regulating tumour survival in a VEGF-dependent manner [105]. As reviewed in Chung et al. 2004 [106], VEGF is also known to have a non-angiogenic function important in evading apoptosis and promoting survival of breast cancer cells. During hypoxia, α6β4 and α6β1 ligation stimulate VEGF production, which in turn functions in an autocrine manner to promote survival signals [107, 108]. Furthermore, in breast cancer and other cancers, β1-integrin has been found to have an aberrant expression that can lead to tumour progression and invasion. Targeting this molecule with a specific β1-integrin antibody to block its interaction with the ECM can abrogate invasive ability [109], and recently it was shown that blocking β1-integrin receptors affected only malignant cells and not normal non-malignant cells [110]. This may be a promising indicator of the potential usefulness of anti-β1-integrin strategies in breast cancer treatment.

Other important ECM receptors for migration and invasion in breast cancer cells include members of the *CD44* family. CD44 is the main receptor for hyalur-

onan (HA) in the matrix, and is present on epithelial cells in different isoforms. CD44 s, the standard isoform, has been shown to be down-regulated in tumours and seems to attenuate metastatic invasion [111]; therefore, its retention is a favourable prognostic factor in patients with node-negative invasive breast carcinomas [112]. Several other isoforms are described to be upregulated in breast cancer and have been considered markers of metastasis and unfavourable prognosis [113–117]. For example, CD44v3 and CD44v10 overexpression appears to play an important role in breast cancer progression [114,115]. In particular, CD44v3 associates in the plasma membrane with MMP-9, and localises at invadopodia, thus highlighting its importance in breast tumour cell migration [118]. Yet another CD44 isoform, CD44v6, may have independent prognostic value for survival or at least be closely related to tumour cell differentiation status [119–121].

Cell-cell adhesion proteins

There are several multi-protein complexes which link cells at the cell-cell interface, namely tight junctions (TJs), adherens junctions (AJs), desmosomes, and gap junctions (GJs). TJs are localized just beneath the apical surface of epithelial and endothelial cells, controlling the passage of ions, water and other molecules between cells and maintaining cell polarity [122]. TJ proteins have only recently been described as key proteins in the progression of breast cancer to an invasive phenotype. Some of the most important TJ proteins to emerge in this context are Claudins, a family of 17–27 kDa integral membrane proteins that form the backbone of tight junctions [123], which have been shown to undergo loss or gain of expression. Claudin-1 is down-regulated at the mRNA and protein level in breast cancer cell lines [124, 125], and in hereditary and sporadic breast cancers [126]. Re-expression of Claudin-1 in breast cancer spheroids induces elevated apoptosis, probably due to reformation of strong TJs in cancer cells such that only a regulated flux of growth factors and nutrients can reach the cells [127]. Another protein of this family involved in breast cancer, Claudin-7, undergoes a loss of expression in ductal carcinomas *in situ* and in invasive ductal carcinomas directly correlating with tumour histologic grade [128–130]. Furthermore, Claudin-4 expression seems to decrease in grade 1 invasive carcinomas, and is reportedly absent in rarer types of breast carcinomas (mucinous, papillary, tubular) and apocrine metaplasia, compared to normal tissue. This could suggest a role for Claudin-4 in cellular differentiation [125].

Zonula Occludens (ZOs), cytosolic linker proteins in breast cancer cells which cooperate in maintaining the integrity of tight junctions, have recently been shown to delocalise away from cell-cell junctions in invasive carcinomas. According to Polette et al. [131], ZO-1 does not localise at the tight junctions in invasive breast carcinomas, and this correlates with the absence of occludin and increased expression of the metalloproteinase MT1-MMP [132]. Furthermore, in breast tumours, loss of ZO-1 expression has been found to correlate with loss of E-cadherin expression and low levels of tumour differentiation [133].

*AJ*s are mainly comprised of classical cadherins and catenins (non-classical cadherins and other specific catenins are involved in desmosome formation as reviewed in Knudsen, 2005) [134]. They are widely involved in breast cancer progression, with E-cadherin being one of the main players. E-cadherin is generally expressed on normal epithelial cells, and in the context of cancer prevents tumour cells from breaking away and invading the surrounding tissue [134]. Furthermore, its presence promotes cell differentiation and growth suppression; and several studies (both cultured cells and mouse models) have defined E-cadherin as a tumour and invasion suppressor. The loss of E-cadherin expression is a defining feature of lobular breast carcinoma *in situ*; however, breast ductal carcinoma *in situ* retains its expression at reduced levels [135,136] (see other implications in the EMT section). During tumour progression and invasion, cells show alterations in cadherin expression, with E-cadherins being down-regulated and N-cadherins upregulated in parallel. N-cadherin, usually expressed by mesenchymal cells, is also expressed on metastatic tumours, and due to an affinity for stromal and endothelial cells seems to facilitate invasion and metastasis [137]. A different cadherin, P-cadherin, generally expressed on myoepithelial cells but not normal epithelial cells, has been found expressed in ductal carcinomas [138] and correlates with higher tumour grade (poorer differentiation). Together with cytokeratin 5, P-cadherin is a putative marker to characterise and distinguish basal-like DCIS [139]. H-cadherin, a newly characterised cadherin molecule, has been shown to decrease its expression in a variety of human carcinoma cells, and this suggests that it may play a role in maintaining a normal cellular phenotype. It has also been found to be down-regulated in breast cancer [140] through promoter methylation [141, 142].

In their intracellular domain, cadherins interact with catenins, a group of sub-membranous proteins that mediate binding to the cytoskeleton. α-, β-catenin and p120 have been described as playing a major role in breast cancer progression. In particular, β-catenin and p120 are involved in cell adhesion but also interact at the nuclear level with transcription factors to alter

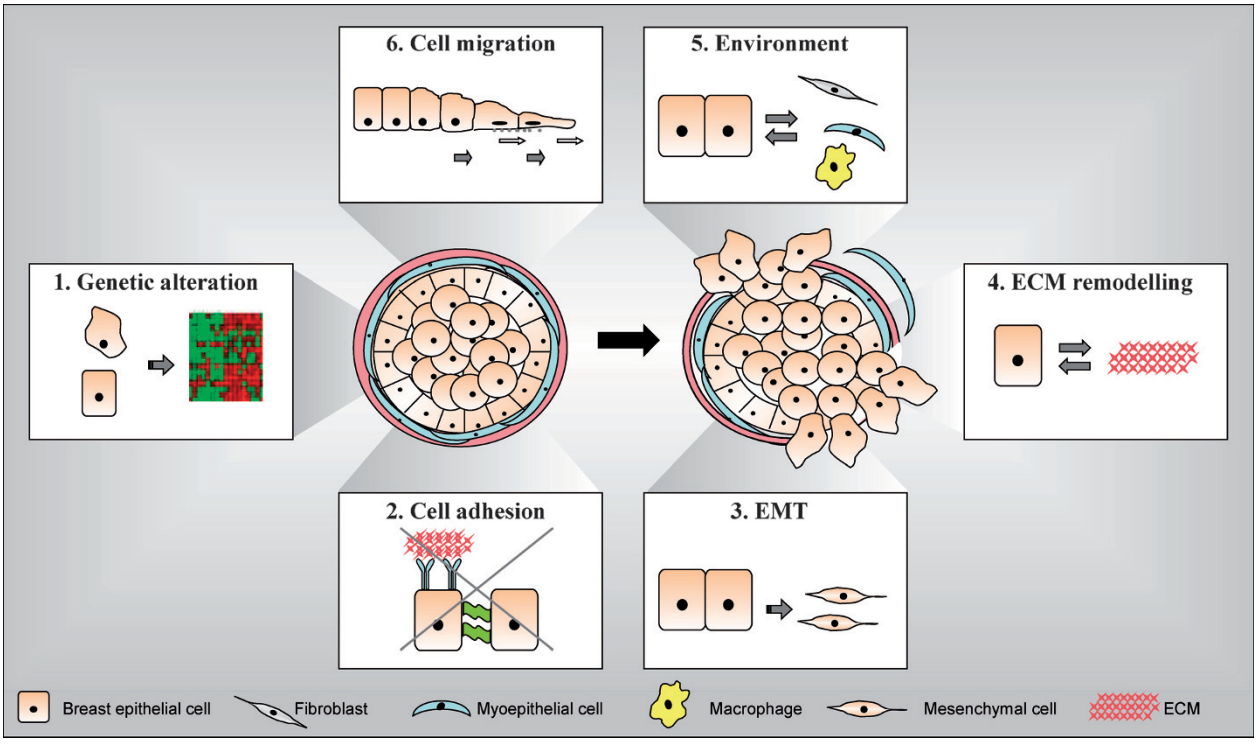

Figure 4. Factors influencing and facilitating breast cancer invasion. Breast cancer arises from the accumulation of genetic alterations (1) The loss of cell adhesion (2) and the process of epithelial-mesenchymal-transition (3) may facilitate the release of cancer cells from a normal tissue architecture. This, along with extracellular-matrix remodelling (4) and influences from the tumour microenvironment (5), may culminate in cell migration (6) The coordination of many of these processes is likely to be required for breast cancer progression and invasion.

gene expression through the Wnt pathway [143, 144]. Several studies have shown β-catenin upregulation in breast tumours and in addition upregulation of β-catenin target genes such as cyclin D1 [145], Twist [146], and CD44 [147]. Finally, genes coding for proteins that destabilize β-catenin have been found to be down-regulated in breast cancer [148], while genes coding for proteins that promote its stability have been found to be up-regulated (like PTEN, ILK, IKKα, IKKβ, Pin, and p53, reviewed in [144]. Together, these mechanisms result in an enhancement of β-catenin transcriptional activity in breast cancer progression and invasion.

Further studies on these proteins will allow a more comprehensive review of their behaviour and contributions to tumour progression, thereby ultimately defining candidate breast cancer prognostic protein markers. Moreover, the study of compounds able to specifically target and block the action of adhesion proteins involved in cancer invasion could be considered as treatment to prevent invasion in breast cancer patients.

Epithelial-mesenchymal transition

As discussed above, cell-cell and cell-matrix adhesion play an important role in normal tissue architecture, and their loss has been associated with invasion. However, in recent years they have also assumed a new significance in the process of epithelial to mesenchymal transition (EMT).

EMT is a component of several normal morphogenic and organogenic processes during embryogenesis. However, this event has also been described in pathological situations. For example, the first step in invasion and metastasis includes the dissociation of adherens junctions, loss of cell polarity, separation into individual cells, and acquisition of cell motility through a program that resembles EMT [149, 150]. Loss of E-cadherin expression through methylation of its promoter in epithelial cells [151] is an early sign of carcinoma progression and unfavourable prognosis [152, 153]. The E-box repressor Snail plays an important role in downregulating E-cadherin expression in EMT [154]. SIP1 (ZEB-2), another factor related to Snail, is also involved in this repression [155, 156]. In addition, the pro-inflammatory factor NF-κB was recently described to induce EMT through activation of ZEB1/ZEB2 and downregulation of E-cadherin expression [157]. *De novo* expression of N-cadherin is also involved in EMT in breast cancer; however, the mechanisms are still unknown [158, 159].

Several signal transduction pathways have been described to promote EMT *in vitro* in epithelial cells. In many cases the MAPK cascade is implicated in EMT in breast. In addition, TGF-β signalling is implicated in EMT pathways through the activation of MAPK and PI3-K [160]. The Src kinase family is also involved, and studies suggest that Src inhibitors may decrease the development of metastasis in ER-positive breast cancers [161]. Integrins and growth factor receptors are also involved in facilitating TGF-β-mediated EMT activation [162]. More detailed aspects of the different factors implicated in EMT in cancer are reviewed in Thiery 2006 [163].

Many of the pathways that cause EMT in cancer have still to be elucidated. A better knowledge of EMT pathways and of the genes involved in EMT in breast carcinomas could be a useful prognostic factor in breast cancer patients.

Conclusion

In this review, we have discussed the processes and changes that influence breast cancer invasion (Fig. 4). We have shown that genetic changes conferring growth advantages to cells, and the breakdown in fundamental processes such as cell adhesion, proteolysis, and cytoskeletal rearrangements, all contribute to breast cancer invasion. Future work in this area may shed more light on key genes and proteins that function in conferring invasive capabilities to cells. Genes or proteins identified may enable further classification of distinct disease subtypes and be useful as diagnostic or prognostic targets. Ultimately, this information will facilitate the development of new and targeted drugs which delay or prevent the process of breast cancer invasion, thereby improving survival outcomes for many women suffering from this disease around the world.

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