Molecular alterations in metaplastic breast carcinoma

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ABSTRACT

Metaplastic carcinoma of the breast is a rare and heterogeneous subtype of breast carcinoma with a generally poor outcome, and few therapeutic options once disease recurs or progresses. Metaplastic carcinomas of the breast are usually of a larger size at diagnosis, with less frequent nodal metastasis compared with invasive ductal carcinoma no special type, and lack hormone and HER2 receptor expression. Recent research has revealed some potentially actionable genetic changes in a subset of these rare tumours. However, ongoing efforts to further characterise the genetic basis and the molecular alterations underlying the distinctive morphological and clinical characteristics of these tumours are needed in order to identify new targets for treatment. This review will describe the theories of pathogenesis of metaplastic breast carcinoma, and highlight genetic changes and potential therapeutic targets in this generally poor prognosis malignancy.

INTRODUCTION

Metaplastic breast carcinoma (MBC) is a heterogeneous group of malignant tumours, and overall comprises <1% of all breast carcinomas.[1] MBC encompasses tumours with entirely epithelial as well as mixed epithelial and mesenchymal components, and may be purely metaplastic or admixed with other types of invasive carcinoma. Tumour cells may show differentiation towards squamous epithelium or mesenchymal elements with spindle, chondroid, osseous and rhabdoid cells,[2] and most are oestrogen receptor (ER), progesterone receptor (PR) and HER2 negative.[3–5] There is no standardised optimal treatment regime for these tumours which overall have a worse prognosis than similar stage invasive ductal carcinoma no special type (IDC NST),[1 6–9] with the probable exceptions of low grade fibromatosis-like carcinoma and low grade adenosquamous carcinoma.[1 4 6] Research into MBC has been limited by the rarity of this tumour and the variety of tumour types included in this diagnosis.

Clinical features, treatment and outcome

Patients with MBC present at a mean age between 48 and 61.1 years[5 10] with a breast mass.[11] These patients present with higher American Joint Committee on Cancer (AJCC) stage, and have a poorer outcome, lower 5-year survival rates and a significantly worse disease free survival than IDC NST.[1 7 9]

Standard chemotherapeutic regimes for IDC NST do not appear to be effective for MBC.[7 12] Nagao *et al*[13] found that disease free and overall survival was worse for patients with MBC compared with IDC NST in the neoadjuvant setting. Adjuvant radiation may improve overall and disease specific survival for patients receiving lumpectomy, or mastectomy performed in 'high risk patients' (defined as tumours >5 cm or four or more metastatic lymph nodes).[14]

Histological and immunohistochemical features

The most recent WHO classification[2] has morphologically classified MBC as low grade adenosquamous carcinoma, fibromatosis-like metaplastic carcinoma, squamous cell carcinoma, spindle cell carcinoma, metaplastic carcinoma with mesenchymal differentiation and mixed metaplastic carcinoma (figure 1A–D). The extent of metaplasia may vary from microscopic foci to a tumour entirely composed of metaplastic elements.

Greater than 90% of MBC are ER, PR and HER2 negative.[4 5 8 15] Immunohistochemistry for 'basal-like markers' including CK14, CK5/6 and epidermal growth factor receptor (EGFR) is often positive (figure 2A,B).[4 15 16] p63 has been reported by several authors to be positive in greater than 90% of MBC and can be useful in the differential diagnosis with other spindle and mesenchymal tumours.[15 17 18]

The differential diagnosis of MBC depends on the histological features of the tumour. Bland spindle cell tumours raise a differential diagnosis including scar, fibromatosis, nodular fasciitis and myofibroblastomas. MBC with more cytological atypia raise a differential diagnosis including malignant phyllodes tumour and primary and metastatic sarcomas.[1 19]

PATHOGENESIS OF MBC

Although the pathogenesis of MBC is unknown there are a number of theories to explain the intratumoural phenotypic diversity of these tumours. These theories include both genetic and non-genetic mechanisms such as transformation of the carcinomatous component into the sarcomatous component through epithelial to mesenchymal transition (EMT); origin of both components from cancer stem cells/multipotent progenitor cells; or origin from myoepithelial cells or myoepithelial progenitors.[3 20 21]

Epithelial to mesenchymal transition

EMT occurs during normal embryonic development via downregulation of E-cadherin function, resulting in epithelial cells acquiring a fibroblast-like phenotype and developing tissue invasive properties.[22] E-cadherin most likely plays a role in the

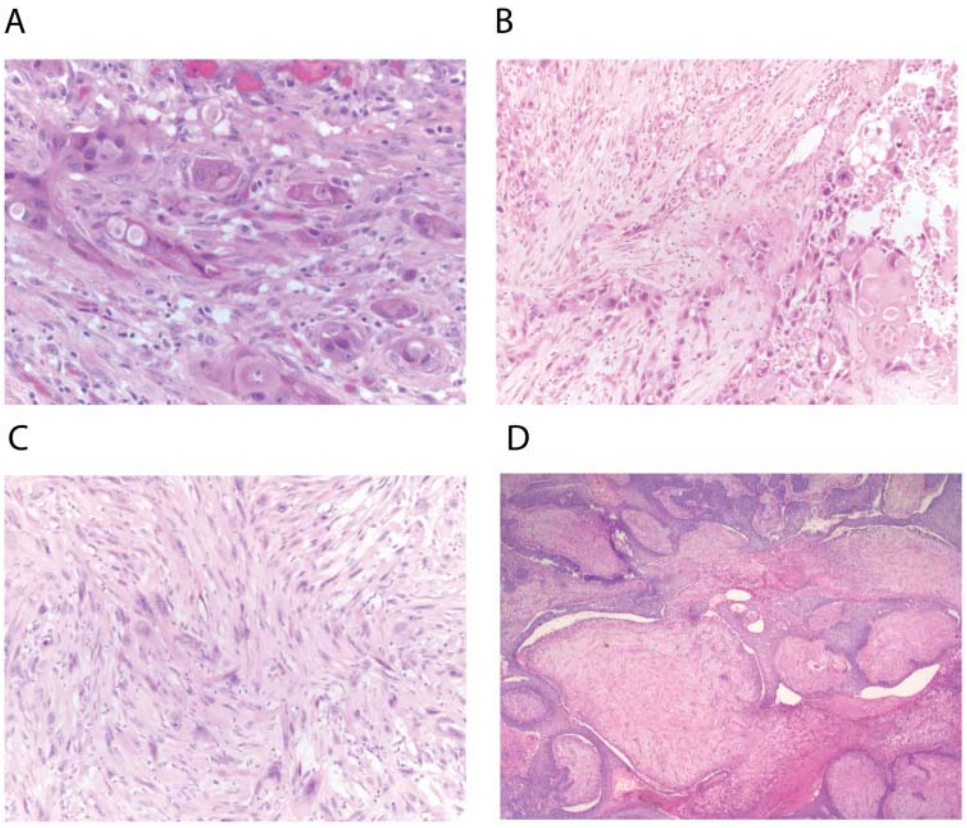

Figure 1 Examples of metaplastic breast carcinoma. (A) Metaplastic carcinoma with squamous features (H&E, 200×). (B) Metaplastic carcinoma with spindle and squamous cell carcinoma (H&E, 100×). (C) Metaplastic spindle cell carcinoma (H&E, 100×). (D) Carcinosarcoma (H&E, 12.5×).

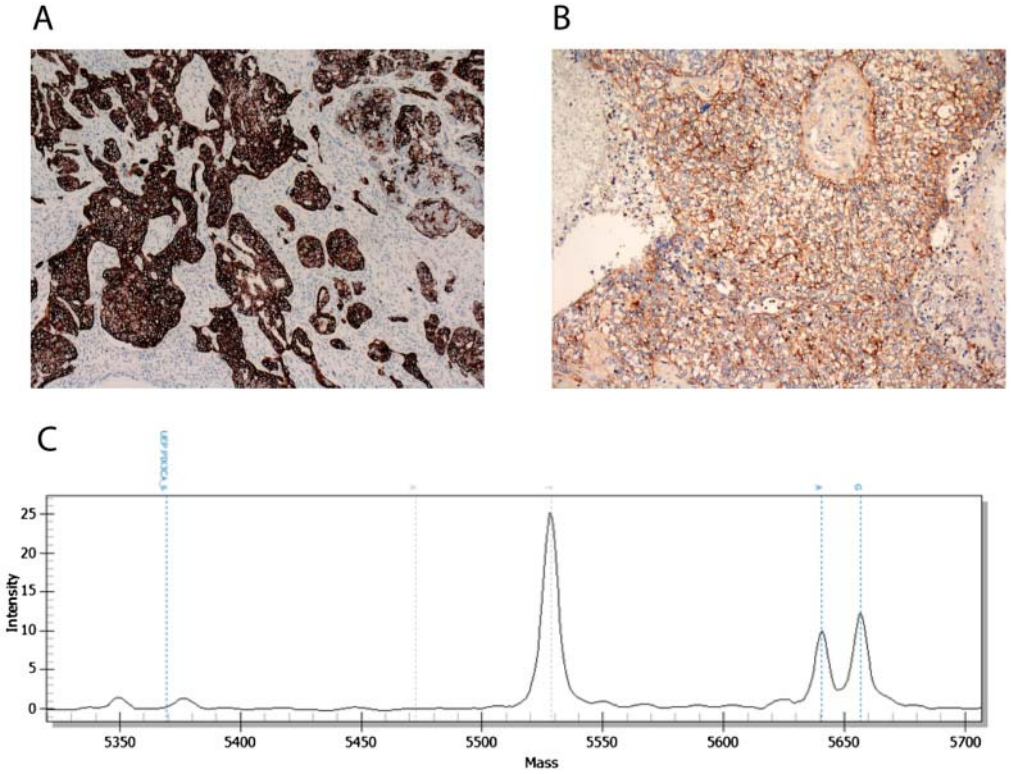

Figure 2 Immunohistochemistry for basal markers in a case of carcinosarcoma (see figure 1D). (A) CK5/6, 40×. (B) Epidermal growth factor receptor, 40×. (C) Sequenom MassARRAY trace showing a second mutant DNA peak (A) consistent with a PIK3CA mutation (G=wild-type peak), in a case of invasive ductal carcinoma with spindle and squamous cell carcinoma (see figure 1B).

abnormal manifestation of EMT in epithelial-derived cancer cells via binding of the zinc finger transcription factor Snail to the promoter region. E-cadherin loss frequently occurs early in MBC along with activation of the Wnt signalling cascade.[22] β-Catenin participates both in E-cadherin dependent adhesion and in Wnt signalling.[23]

EMT is characterised by the loss of epithelial characteristics and acquisition of mesenchymal characteristics. EMT occurring after embryogenesis is considered pathological and is a mechanism of carcinoma progression and metastasis.[22 24] There is loss of intercellular adhesion (mediated by E-cadherin and occludins), downregulation of epithelial markers (including cytokeratins), upregulation of mesenchymal proteins (vimentin and smooth muscle actin (SMA)), acquisition of fibroblast-like spindle cell morphology, as well as increase in motility, invasiveness and metastatic potential.[24 25] 'Cadherin switching' and accumulation of β-catenin have also been associated with EMT.[24 26] EMT is mediated by specific transcription factors including Snail, Slug, SIP-1, dEF1, E12/E47 and Twist, which act as transcriptional repressors of E-cadherin and modulate expression of genes involved in cancer invasion and metastasis.[24] Snail also has a function in blocking the cell cycle and in resistance to cell death.[27]

The theory of EMT has been supported by gene expression profiling (GEP) studies of MBC, which have found overexpression of genes related to adhesion, motility, migration and extracellular matrix formation, and downregulation of genes encoding proteins. The GEP of MBC was found to be distinct to IDC NST. The EMT core signature includes upregulation of Snail, Twist, transforming growth factor-β (TGF-β) and Goosecoid and downregulation of E-cadherin, which is found both in MBC and claudin-low tumours.[21 28] Reflecting this finding, E-cadherin and Snail expression by immunohistochemistry have an inverse relationship, with nuclear Snail expression and loss of E-cadherin seen in metaplastic chondroid cells in a series of MBC.[29]

The mechanisms responsible for triggering EMT are unknown; in vitro EMT can be induced by extracellular matrix components and growth factors.[24 25] TGF-β pathway activation with upregulation of the EMT inducers Snail, Slug, Twist and Zinc finger E-box-binding (ZEB) lead to complete EMT in some cancer models.[24] Storci *et al*[30] found that Slug mRNA is upregulated in a proportion of basal-like breast cancer (BLBC), with Slug being the most likely factor responsible for EMT-like properties of these tumours.

Snail at the transcriptional levels upregulates Moesin, a membrane–cytoskeleton linking protein that plays a role in cell morphology, adhesion and motility. Moesin is an immunohistochemical marker of EMT and is highly expressed in MBC, predominantly in the sarcomatous component (95.7% of 23 cases), in comparison with IDC NST (16% of 244 cases). Moesin expression has also been demonstrated in 81.5% of extramammary carcinosarcomas.[31] High Snail expression in breast cancers has been associated with a poor relapse free survival in other types of breast carcinoma and predicts disease free survival.[32] Immunohistochemistry for Snail has been shown to be a sensitive, but not specific diagnostic marker of MBC, as it is also seen in other spindle cell lesions of the breast including myofibroblastoma and phyllodes tumour.[33]

Stem cell-like characteristics

The breast cancer stem cell (BCSC) hypothesis suggests that a single tumour-initiating cell with stem-like characteristics is responsible for breast cancer. BCSC have a CD24 (negative/low) CD44 (positive/high) phenotype, and are seen in higher frequency in BLBC.[25] High CD44/CD24 and CD29/CD24 ratios have been identified in MBC, consistent with a high level of stem cell-like cells in these tumours.[26] ZEB1 is progressively overexpressed and E-cadherin downregulated in MBC, both features of EMT, and at the same time the tumours acquire BCSC marker proteins ALDH-1 and CD44 (high)/ CD24 (negative/low).[34]

There is a direct link between EMT and gain of BCSC properties.[35 36] EMT inducers including TGF-β, Snail and Twist confer a stem cell phenotype in immortalised human mammary epithelial cells.[25] Induction of EMT in immortalised human mammary epithelial cells by Twist or Snail transcription factors resulted in the cells acquiring mesenchymal traits and the expression of stem cell markers CD44 (high)/ CD24 (negative/low).[35] It is unclear whether any of the EMT inducers are involved in breast stem cell differentiation in vivo.[24] BCSC are also thought to be resistant to chemotherapy,[37] and this may contribute to the chemoresistance of MBC.

Myoepithelial cell origin

The myoepithelial cell origin theory of MBC arose from morphological observations including circumferential proliferation of tumour cells around ducts, and metaplastic changes which have also been observed in reactive myoepithelial cells and other myoepithelial cell tumours.[18] Several immunohistochemical markers found in myoepithelial cells of the normal breast, including p-cadherin, p63, mapsin, CD10, SMA, S100 and glucocorticoid receptor, among others, have been shown to express preferentially in MBC.[18 38 39] GEP has also found that MBC overexpress some genes found in normal myoepithelium of the breast including p-cadherin, p63 and calponin.[3 15 39] A recent animal study has suggested that CD10 positive myoepithelial/basal cells are likely the source of MBC.[40]

While there are data supporting each of these hypotheses there is no widespread acceptance of any one of these as the underlying cause of MBC. What is more critical is identifying potential therapeutic targets and much work has been done using genetic techniques such as GEP, array comparative genomic hybridisation and in situ hybridisation, and investigating specific signalling pathways such as phosphatidylinositol 3-kinase (PI3K), p53, Wnt and EGFR.

Genetic analysis of MBC

Using array comparative genomic hybridisation MBC show differences in DNA copy number gains and losses, a high level of genomic instability, and a unique set of genetic aberrations compared with IDC NST.[26] MBC show gains of distal chromosome 1p/5p and loss of 3q;[26] in contrast, alterations common in IDC NST such as gain of 1q and loss of 16q[41 42] are uncommon in MBC. MBC also show different genetic alterations to BLBC; in particular, *PIK3CA* mutations were detected in 47.4% of MBC compared with 8.3% of BLBC.[26] These results identified in a small number of cases suggest that MBC represent a distinct subgroup of breast cancer.[26]

Genetic studies support a monoclonal origin of the heterogeneous components of MBC, demonstrating that histologically distinct components are clonally related in the majority of cases,[20 43 44] including common p53 mutations in carcinomatous and sarcomatous components.[43] Geyer *et al*[20] also demonstrated two cases with additional genetic alterations in metaplastic areas, and one case with additional genetic changes in the nodal metastasis, implying that morphological diversity may be due to progressive genetic alterations.

MOLECULAR FEATURES OF MBC

PI3K signalling pathway

The PI3K signalling pathway impacts on cell growth, survival and metabolism, including a central role in mediating the effects of insulin on cellular metabolism. PI3K signalling is activated in cancers by direct mutational activation or amplification of genes encoding key components of the PI3K pathway (eg, *PIK3CA*, *AKT1*), or loss of the tumour suppressor phosphatase and tensin homologue deleted on chromosome 10 (*PTEN*). This results in alterations of cancer cell growth, survival, motility and metabolism. Inhibition of PI3K signalling can reduce cell proliferation and promote cell death, as well as possibly affecting tumour angiogenesis and metastasis.[45]

The most common genetic alteration of PI3K signalling found in human cancer is *PTEN* inactivation resulting in loss of its lipid phosphatase activity. Most somatic mutations lead to protein truncation; however, there are also missense mutations. In addition, *PTEN* inactivation can occur through transcriptional repression and epigenetic silencing via promoter hypermethylation. Somatic mutations of *PIK3CA* have been described in breast cancers as well as other malignancies such as colonic carcinoma, endometrial carcinoma and glioblastoma, with most mutations clustering to exons 9 and 20. These mutations lead to constitutive signalling of the PI3K pathway in the absence of growth factors. Expression of mutated *PIK3CA* in mammary epithelial cells leads to transformation, growth factor independent proliferation and resistance to apoptosis.[46] *PIK3CA* mutations and *PTEN* inactivation appear mutually exclusive.[47]

Mutations in key genes of the PI3K pathway have been well described in IDC NST. In their study of 133 primary breast carcinomas, Saal *et al*[47] found a correlation among *PIK3CA* mutations and ER positive, PR positive, lymph node positive breast cancer as well as a positive association with HER2 overexpression, whereas ER negative PR negative breast cancer more commonly associated with *PTEN* mutations. Stemke-Hale *et al*[48] also found *PIK3CA* mutations more commonly in hormone receptor positive and HER2 positive tumours than in BLBC. Specific studies addressing MBC are rare: Hennessy *et al*[26] found that PI3K pathway gene mutations are present in up to 47% of MBC, suggesting PI3K pathways changes may play an important role in MBC. This finding also highlights that targeting the PI3K pathway may be a promising therapeutic target (see later section) (figure 2C).

p53 Protein

p53 is a tumour suppressor protein encoded by the *TP53* gene, which is located on 17p13.1. It regulates the cell cycle at G1/S, initiates apoptosis, inhibits of angiogenesis and can activate DNA repair.[49 50] Mutation or deletion of the *TP53* gene is common in human cancers (seen in up to 50%), reducing tumour suppressor function.[43] *TP53* is altered in approximately 20%–40% of all breast carcinomas, particularly triple negative breast cancers, and appears to be an early event in tumourigenesis. Most *TP53* alterations are point mutations, which in most cases cause production of a stable and malfunctioning protein accumulating in tumour cells. This can be detected by immunohistochemistry, although this is not entirely specific.[49] However, less than 75% of cases with *TP53* mutations detected by sequencing have p53 protein detected by immunohistochemistry, possibly due to some mutations causing an unstable truncated protein, or the secondary accumulation of wild-type p53 due to DNA damage.[50]

p53 protein overexpression analysed by immunohistochemistry has been demonstrated in MBC with squamous differentiation (66% of six cases)[51] and in up to 61% of MBC, compared with 20%–40% of IDC NST.[49] Lien *et al*[43] found p53 overexpression in 71% of 14 cases of MBC, but p53 mutations identified by direct sequencing were only found in four of the 10 cases, possibly due to decreased sensitivity of Sanger sequencing on formalin fixed, paraffin embedded material. These authors found the same p53 mutations in the carcinomatous and sarcomatous components, as well as in one case in the in situ carcinoma, suggesting that early p53 mutation is maintained throughout tumour progression.[43] Geyer *et al* had similar results with three of six cases of MBC showing p53 overexpression by immunohistochemistry, and only two of the cases with p53 mutations detected by direct sequencing. There were identical p53 mutations in the morphologically distinct components.[20] Hennessy *et al*[26] found p53 mutations in 32% of 19 MBCs.

A recent study showed that in breast carcinoma *TP53* mutation involves derangement of other pathways, including vascular endothelial growth factor (VEGF) signalling pathway, mitogen-activated protein kinase (MAPK) and calcium signalling, although the survival effect of VEGFA was found only in ER positive tumours.[52] High levels of the angiogenesis markers VEGF and hypoxia inducible factor (HIF)-1α have been observed in metaplastic carcinomas, particularly in MBC with squamous differentiation.[51 53 54]

Wnt signalling pathway

The Wnt pathway is a cell signal transduction pathway consisting of a network of separate but interacting pathways that influence embryonic development, cell proliferation, cell polarity, cell adhesion, apoptosis and tumourigenesis.[55 56] In the canonical pathway, specific Wnt ligands bind to their target transmembrane receptors to inhibit the multi-protein destruction complex, resulting in downstream activation of gene transcription by β-catenin,[56] modulating genes such as *c-MYC* and *CCND1* encoding cyclin D1.[57] Wnt signalling is involved in growth and differentiation of the mammary gland.[56]

Mutations in Wnt pathway genes including β-catenin and adenomatous polyposis coli (*APC*) causing hyperactivation of the Wnt/β-catenin pathway are well described in many forms of human cancer[58] but are rare in breast cancer.[56] Animal studies have shown that endogenous β-catenin gene mutation results in squamous metaplasia in the mammary gland,[59] and that Wnt pathway activation plays a central role in EMT.[23] Elevated β-catenin expression in human breast cancer correlates with overexpression of cyclin D1 and has been associated with poor clinical outcome in breast cancer.[56 60]

One study has shown aberrant expression of β-catenin in 33 out of 36 MBC. In all, 25% of these showed missense mutations of the β-catenin (*CTNNB1*) gene encoding the NH2-terminal portion of β-catenin, two cases showed inactivating mutations in the *APC* gene and five showed frame shift mutations of the Wnt-1 induced secreted protein 3 gene (*WISP3*). Both *CTNNB1* and *APC* mutations were more common in the mesenchymal components; however, *WISP3* mutations were more common in the epithelial cells.[58] Mutations of *CTNNB1* and *APC* genes lead to increased levels of β-catenin and Wnt pathway activation and have been shown to promote tumour development in colon cancer.[61] *WISP3* belongs to the *CCN* family of growth factors mediating stromal and epithelial cross talks, and deregulation can lead to cancer[58] as well as causing EMT and triggering motility and invasion in human mammary epithelial cells.[62]

In contrast, Lacroix-Triki *et al* found membranous β-catenin expression in 49 of 52 cases of MBC, with 23% showing nuclear expression (a surrogate marker of Wnt pathway

activation). None of the MBC cases showed exon 3 *CTNNB1* mutations by direct gene sequencing, suggesting that aberrant β-catenin expression in MBC may be due to mutations affecting other exons of the *CTNNB1* gene or other genes in the Wnt pathway, or that Wnt pathway activation may occur by other means.[57] A further study by Geyer *et al*[63] also failed to demonstrate *CTNNB1* mutations in a large cohort of primary invasive carcinomas including a small number of MBC.

Epidermal growth factor receptor

The *EGFR* gene is located on chromosome 7p11.2 and encodes a transmembrane tyrosine kinase receptor. Once activated by growth factor ligands EGFR becomes dimerised, stimulating intrinsic intracellular protein tyrosine kinase activity via autophosphorylation of intracellular tyrosine residues, resulting in downstream activation of signalling initiating several signal transduction cascades including the MAPK, Akt and cJun N-terminal kinase (JNK) pathways. This results in DNA synthesis and cell proliferation, and modulation of cell migration, adhesion and proliferation. *EGFR* gene mutations cause ligand independent receptor tyrosine kinase activity and constitutional activation of cell proliferation and cell survival signalling pathways, which can cause neoplastic transformation of cells.[64]

EGFR overexpression has been identified in up to 80% of MBC, with up to 23%–37% of these cases showing *EGFR* amplification detected by in situ hybridisation.[16 65] *EGFR* amplification was found particularly in tumours with squamous or spindle cell differentiation.[16 66] No activating mutations in exons 18–21, the hot spot for *EGFR* gain of function mutation and the target for the tyrosine kinase inhibitor gefitinib, have been found in MBC.[16 66] The mechanism for EGFR overexpression is unknown,[16 65] although Gilbert *et al*[66] demonstrated high copy number of *EGFR* primarily via aneusomy, which may indicate a role for treatment of MBC by EGFR tyrosine kinase inhibitors. Hennessy *et al* found that components of three major branches of the MAPK signalling pathway are overexpressed in MBC including JNK, MAPK and p38, with phosphorylation of three of four protein components of these pathways (JNK, mitogen-activated protein kinase kinase (MEK), p38) higher in MBC compared with IDC.[26]

α β-Crystallin

α β-Crystallin (ABC) is a member of the mammalian small heat shock protein family and is also a structural protein. It inhibits apoptosis, suppressing the autocatalytic maturation of caspase-3[67] and preventing the mitochondrial translocation of the pro-apoptotic Bcl-2 proteins Bax and Bcl-x_s.[68] It also has a cytoprotective effect, functioning as a molecular chaperone to inhibit intracellular aggregation of denatured proteins[69] and can be induced by stressors including chemotherapy, reactive oxygen species and heat shock.[70] ABC overexpression in xenograft models is tumourigeneic, inducing both mesenchymal-like spindle elements and atypical glandular elements with invasive properties, which may indicate a role in EMT.[71]

ABC is expressed in 45% of BLBC compared with 6% of non-basal carcinomas, and predicts poor clinical outcome in breast cancer patients independent of tumour grade, lymph node, ER or HER2 status.[71] Immunohistochemical expression of ABC was found in 41% of 29 cases of MBC, mainly within the epithelial component as well as in the spindle cell or sarcomatous component in five cases,[69] compared with 56% of BLBC, and no ER positive or HER2 positive carcinomas, a finding which has been replicated in some,[72] but not other studies[70] (the latter including only five cases of MBC).

Laminin 332

Laminin 332, which functions in binding epithelial cells to the basement membrane, and migration of epithelial cells during wound repair, has been found to induce invasion in breast cancer cells in vitro. It has been identified immunohistochemically in 96% of MBC, with the strongest expression at the tumour– stroma interface, in infiltrative nests of tumour and at the invasive front of tumour nodules. MBC with nodal metastasis also had significant higher levels of laminin 332 expression. It is uncertain whether laminin 332 expression causes more aggressive behaviour of the tumour cells, and whether it can predict prognosis.[73]

Caveolin-1

Caveolin-1 is an integral membrane protein, a component of caveolar membranes found in the plasma membrane of most mammalian cells. The gene maps to 7q31.1. It has been reported to play a role in lipid transport, membrane trafficking, gene regulation and signal transduction, and has been suggested as a tumour suppressor gene. One study found that caveolin-1 is expressed in normal myoepithelial cells, as well as in breast carcinomas with basal/myoepithelial differentiation; 35 of 39 cases of MBC were found to overexpress caveolin-1 by immunohistochemistry, with one showing gene amplification by in situ hybridisation.[74]

IS MBC A BLBC?

Transcriptional profiling has defined subtypes of breast cancer, including luminal A, luminal B, Her2 amplified and Basal-like.[75 76] BLBC are a heterogeneous group of tumours characterised by high grade nuclei, high mitotic rate, necrosis, pushing border, central scar or necrosis syncytial growth and a brisk lymphocytic response. BLBC also show frequent node positivity, a high incidence of recurrence, distant metastasis and poor survival, and there is a lack of targeted therapies for these tumours.[69 77]

There is no currently accepted definition of BLBC,[77–79] with some groups using expression profiling and others using immunohistochemical markers as surrogates. Most BLBC lack ER, PR and HER2 overexpression, and express genes and proteins found in basal cells of the normal breast including high molecular weight cytokeratins, p-cadherin, caveolins 1 and 2, nestin, and ABC.[69–71] p53 Expression or *TP53* gene mutations are seen in up to 85% and alterations of *pRB* and p16 checkpoint are common.[78]

Microarray based GEP has demonstrated that MBC are part of the spectrum of BLBC at the transcriptional level.[3 15 80] The majority of MBC show a basal-like phenotype.[15] However, in contrast to BLBC, which generally have a good response to neoadjuvant chemotherapy, MBC do not have the same response.[3] Hennessy *et al*[26] suggest that MBC are a unique subtype of tumours independent to BLBC and most closely related to claudin-low tumours,[81] although there are differences between these tumour groups including widespread differences in gene expression, and a lack of *PIK3CA* mutations in claudin-low tumours.[37]

IMPLICATIONS FOR TREATMENT

A number of the observed molecular changes found in MBC indicate possible targets for therapy for this group of tumours; the rarity of this tumour requires a large, multicentre trial to investigative the efficacy of any new treatments. Weigelt *et al*[3] have demonstrated downregulated canonical BRCA1 DNA

damage response and G2/M DNA damage checkpoint regulation, as well as *PTEN* and *TOP2A* (the targets of anthracyclines) downregulation in MBC. This may explain the difference in chemotherapy response between BLBC and MBC, and indicate potential therapeutic agents such as platinum salts and poly (ADP) ribose polymerase inhibitors.[82]

Several characteristics of MBC including stem cell-like features, EMT and genomic alterations in the PI3K pathway suggest a mechanism of chemoresistance, with *PIK3CA* mutations conferring resistance to paclitaxel chemotherapy.[46] A number of potential therapeutic agents targeting the PI3K signalling pathway have been developed and some are in clinical trials,[45] including mammalian target of rapamycin (mTOR) inhibitors, small molecular inhibitors of PI3K, dual mTOR–PI3K inhibitors, Akt inhibitors and rapamycin analogues among others (reviewed in[83]). ABC is a predictor of resistance to neoadjuvant chemotherapy in IDC NST due to its multiple cell death antagonist roles[84] as well as a potential target. The carcinoma phenotype induced by ABC is suppressed by inhibitors of the *MEK/extracellular signal related kinase (ERK)* pathway, which is constitutively activated in ABC overexpression.[71] Another novel approach may be blockade of EMT or tumour stem cells,[19] or blocking the β-catenin/Tcf4 pathway.[60]

New chemotherapy approaches

Chien *et al*[85] reported a case of MBC treated with neoadjuvant chemotherapy with bevacizumab–doxorubicin–dacarbazine for the sarcomatous component followed by gemcitabine–paclitaxel for the IDC component, with an almost complete response. One case report of an inoperable, large MBC which was resistant to neoadjuvant chemotherapy, which then used ifosfamide and etoposide to target the sarcomatous features, showed a partial remission.[86] A recent study by Moulder *et al* treated patients with metastatic MBC on a phase I clinical trial of liposomal doxorubicin, bevacizumab (an antiangiogenic agent) and temsirolimus, an mTOR inhibitor with action in the PI3K pathway as well as an HIF inhibitor. Five patients with metastatic MBC were treated with some responses and durable complete remission.[54] The same authors conducted a further study on patients with advanced malignancies including 12 patients with MBC, using bevacizumab, temsirolimus and liposomal doxorubicin. Five of the patients with MBC showed a partial or complete remission. The study also demonstrated that tumours with *PIK3CA* mutations and/or *PTEN* loss or mutation had a better response. These small series demonstrate the potential to treat MBC effectively with sarcoma-like and antiangiogenesis chemotherapy.

CONCLUSIONS

Despite the improvements in chemotherapy for breast cancer over the past decade, MBC still responds poorly to traditional regimes. Innovative treatments, including those targeting the molecular characteristics of this group of tumours, offer promising therapeutic possibilities and should be investigated with multi-institutional prospective studies.

Take home messages

- Metaplastic breast carcinoma (MBC) has a worse outcome than matched triple negative invasive ductal carcinoma.
- Poor prognosis may be due to high initial stage at presentation as well as the intrinsic biology of MBC.
- Newly characterised molecular abnormalities may provide targets for personalised therapy.

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