RCSI smj review

Understanding metastasis: current paradigms and therapeutic challenges in breast cancer progression

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Abstract

Metastasis is the terminal event in carcinogenesis and the principal contributor to mortality in breast cancer patients. However, relatively little is known about the molecular mechanisms of this complex, multi-step process. Consequently, management of metastatic breast cancer (MBC) is far less successful than the treatment of primary disease. Research in the field is very active, resulting in the development of numerous models of metastasis and the identification of key factors implicated in the process. Thus it is hoped that with continued research into the mechanisms driving cancer progression and metastasis, insights will be gained that will facilitate earlier diagnosis and improved treatment of MBC.

Key words: Breast cancer, metastasis, metastatic regulators, targeted therapies.

Abbreviations – **MBC:** metastatic breast cancer; **EMT:** epithelial-mesenchymal transition; **MET:** mesenchymal-epithelial transition; **EGF:** epidermal growth factor; **VEGF:** vascular endothelial growth factor; **PDGF:** platelet-derived growth factor; **RNAi:** RNA interference; **miRNA:** microRNA; **ESMO:** European Society of Medical Oncology; **CTC:** circulating tumour cell.

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Introduction

Breast cancer is the most common female malignancy in the developed world, with over 60,000 women in the EU succumbing to the disease each year.[1] The Irish National Cancer Registry predicts that by 2020, the number of cases in this country could increase from the current yearly average of 1,895 to almost 5,000 cases per annum.[2]

Over the past two decades, the management of breast cancer has evolved dramatically due to an increased awareness of the disease, advances in mammographic screening, improved surgical techniques and the increased use of adjuvant therapy.[3] Current treatment strategies are based on the grade, stage and hormone/growth factor receptor status of the disease. Treatments typically rely on surgery combined with adjuvant radiotherapy and systemic medical therapy, including both chemotherapy and endocrine therapy.[4] Owing to these strategies, the treatment of primary breast cancer is extremely effective when detected at an early stage. Indeed, much of this success can be attributed to novel and targeted therapies that have resulted from years of focused research into the molecular basis of breast cancer. Thus, breast cancer management is the archetypal example of personalised medicine. A brief overview of these therapies is provided in **Table 1**.

However, as with most other malignancies, the main cause of death in breast cancer patients is not the primary tumour but metastases to different sites, with death arising as a result of direct organ damage by the growing lesions, paraneoplastic syndromes or complications following treatment.[5] Despite this fact, relatively little is known about the key molecular mechanisms and determinants driving this terminal stage of the disease. Thus far, no

doctrine akin to Hanahan and Weinberg's "Hallmarks of Cancer"[6] has yet been compiled for the metastatic process. Consequently, current diagnostic and treatment strategies for metastatic breast cancer are much less successful.

Our lack of in-depth knowledge regarding metastasis is partly attributed to the complex and multifaceted nature of the process, which encompasses various dynamic physiological activities. These include invasion of the local tissue and entry into the circulatory or lymphatic systems, which transport the metastatic cells to distant sites where they may extravasate and enter the surrounding microenvironment. At this point, colonisation and continued development is typically dependent on favourable interactions with the secondary microenvironment **(Figure 1)**.[7]

Table 1: Role of molecular research in the development of targeted breast cancer therapies.

Targeted treatment strategies in primary breast cancer

- Endocrine therapy arose from a specific understanding of the role oestrogen plays in mammary gland development and the promotion of breast cancer growth.
- The monoclonal antibody trastuzumab (Herceptin) was developed to treat HER2/neu-positive tumours following the discovery that over-expression of the receptor resulted in constitutive activation and thus increased tumour cell proliferation.[29]
- These tumours may also be treated with the dual HER2/neu and EGFR tyrosine kinase inhibitor lapatinib (Tyverb/Tykerb), which is currently in trials for use both as monotherapy and in combination with other therapeutic regimes.[30]
- A recent addition is PARP inhibitors, which are under trial for the treatment of mutant BRCA1/2 breast tumours. Theses agents inhibit ss DNA repair, which triggers selective tumour cell death when coupled with the cells' existing ds DNA repair deficit.[31]
- Deciding the best treatment strategy can, however, be challenging, especially when it comes to the use of adjuvant chemotherapy in lymph node negative patients. Thus, gene profiling techniques are used to stratify patients on the basis of their predicted prognosis in an effort to guide treatment.
- The two main molecular profiling tools currently available are the RT-PCR assay, *oncotype DX™*, and the DNA microarray assay, *MammaPrint®*, both of which are under investigation in the TAILORx[32] and MINDACT trials,[33] respectively.

Abbreviations – **HER2/neu:** human epidermal growth factor receptor 2 (ERBB2); **EGFR:** epidermal growth factor receptor; **PARP:** poly(ADP-ribose) polymerase; **BRCA1/2:** breast cancer susceptibility gene 1/2; **ss DNA:** single-stranded DNA; **ds DNA:** double-stranded DNA; **RT-PCR:** reverse transcriptase polymerase chain reaction; **TAILORx:** Trial Assigning Individualized Options for Treatment; **MINDACT:** Microarray in Node-Negative Disease May Avoid Chemotherapy.

Classical models of metastasis

One of the first and most enduring metastasis models was proposed in 1889 by Stephen Paget, who was struck by the propensity of some cancers to yield metastatic growths in specific organs. He believed that their distribution was not random, but was the result of the complementary interactions between the tumour cells and host environment. He compared this interaction to that of a seed and the soil stating: "When a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil".[8] The "seed and soil" theory was later disputed by James Ewing, who suggested that the circulatory patterns between primary tumours and ectopic growths were sufficient to account for organ-specific metastasis.[9] However, through a series of autopsy studies, Leonard Weiss lent credence to Paget's theory by demonstrating that the site of many metastatic growths could not be accounted for by mechanical blood flow patterns alone.[10]

New paradigms in metastasis

Metaplasia

More recently, epithelial-to-mesenchymal transition (EMT), and the reverse process of mesenchymal-to-epithelial transition

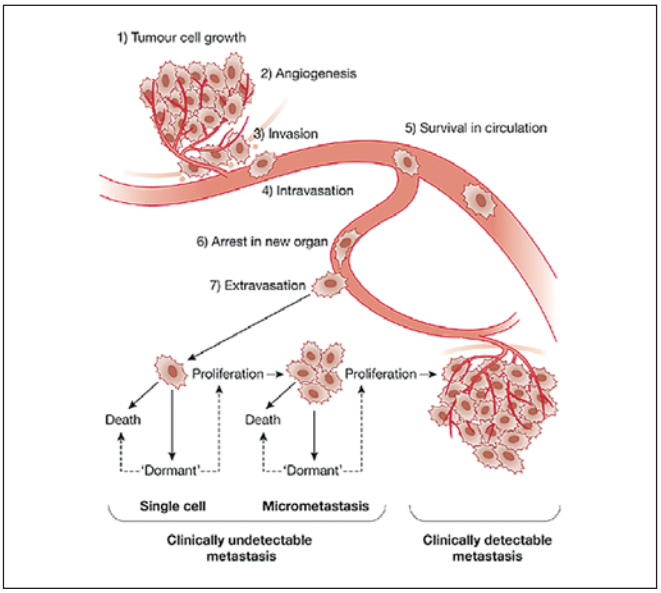

FIGURE 1: Overview of the key molecular events in metastasis. During the metastatic process, cancer cells proceed through a series of distinct, rate-limiting steps to form an overt, secondary tumour. In the initial stages, cells detach from the primary tumour mass, invade adjacent tissue and then enter the lymphatic or circulatory systems, which transport them to distant sites, where they extravasate and enter the surrounding microenvironment. At this point, specific factors determine if the cells may proliferate to form a clinically detectable metastasis or if they are to remain dormant as single cells or micrometastases. (Figure reproduced with authors' permission.[28]*)*

(MET), have been implicated in the dissemination of tumour cells and their subsequent growth at distant sites. EMT describes a series of events during which cells lose many of their classic epithelial characteristics and take on properties typical of mesenchymal cells. Central to this transition is the reduction of cell-cell adherence as a result of the transcriptional repression and delocalisation of cadherins (adherens junctions), occludin and claudins (tight junctions) and desmoplakin (desmosomes). These changes allow the cells to separate and trigger alterations in shape and adhesion, facilitating movement.[11] Cycles of EMT and MET play critical roles in embryogenesis, where they are tightly regulated by various growth/transcription factors and interaction with the surrounding environment. In cancer, EMT is thought to enhance dissemination by facilitating the acquisition of a more motile, invasive phenotype while also protecting the cells in circulation. Conversely, MET is believed to allow diseminated tumour cells to re-establish their epithelial characteristics and facilitate proliferation and colonisation at the secondary site.[12,13]

Microenvironment and inflammation

Of note with regard to the EMT model is the emphasis it places on the surrounding microenvironment, which is thought to initiate and maintain EMT-mediated tumour progression and metastasis. This is reminiscent of Paget's seed and soil hypothesis, a theory that has seen a revival in recent years. Researchers have realised that they may have been too focused on the intrinsic properties of the tumour cell (the seed) and thus failed to recognise the role of the microenvironment (the soil). The importance of this contribution was recently highlighted by Finak *et al*, who successfully used DNA microarray analysis to define a stroma-derived prognostic predictor (SDPP) that could stratify disease outcome independently of standard clinical prognostic factors.[14] Examination of the 26-gene SDPP revealed many encoded proteases, chemokines and cell surface receptors associated with immune cells, emphasising the important role the inflammatory response plays in tumour progression. This observation was recently reinforced by Lisa Coussens and colleagues at UCSF, who demonstrated a metastasis-promoting role for T_H2-CD4$^+$ T-lymphocytes in a pre-clinical mouse model of mammary carcinoma.[15]

Dormancy

Dormancy is another intriguing aspect of metastasis and is particularly common in breast cancer, where between 20 and 45% of patients relapse years or even decades after their initial diagnosis.[16] During this asymptomatic interim, minimal residual disease is thought to exist as pre-angiogenic micrometastases or solitary dormant cells. The latter are typically quiescent, while micrometastases are actively proliferating, their growth balanced by apoptosis and thus resulting in no net increase in size.[17] Dissemination to a non-permissive microenvironment is thought to be an important factor in dormancy. However, the specific physiological and/or pathological influences that determine whether tumour cells remain in this dormant state or actively proliferate to form an overt macrometastasis are not yet known. However, activation of a so-called 'angiogenic switch' is thought to be necessary, whereby the balance between the pro-angiogenic (e.g., VEGF and PDGF) and anti-angiogenic (e.g., thrombospondin and angiostatin) factors necessary to maintain dormancy is tipped in favour of the former.[17] As such, anti-angiogenic therapies may prove beneficial in maintaining disseminated tumour cells in a dormant, sub-clinical state in the future.

Metastatic mediators

It is evident that it will take some time to reconcile the various theories of metastatic mechanisms, such that an integrative model of tumour progression can be developed. However, research has also been focused on the identification of key mediators that could serve as novel points for therapeutic intervention.

These mediators can be classified as either effectors or regulators, where the former are factors that promote or inhibit a specific step in the metastatic cascade (i.e., invasion and migration), but cannot by themselves direct the entire process. However, given that each step in metastasis is rate limiting, the disruption of these effectors should be sufficient to block the formation of distant metastases. Many of the metastatic effectors identified to date inhibit specific stages in the process and are referred to as metastasis suppressor genes. These genes are typically lost or down-regulated in aggressive metastatic cancers and their suppression in preclinical mouse models results in an increase in the formation of metastatic foci. However, unlike tumour suppressor genes, which inhibit tumourigenesis, metastasis suppressors only inhibit metastasis and have no effect on the growth of the primary tumour.[18] Interestingly, many of these genes function by inhibiting the growth of cells at ectopic sites and thus may play a role in dormancy (e.g., KISS1 and BRMS1).[19,20]

The identification of key regulators of metastasis is, however, far more challenging, as these genes must be capable of orchestrating all the disparate processes required for a tumour cell to disseminate and grow at an ectopic site. Nevertheless, a breakthrough was recently made on this front with the identification of the transcription factor SATB1, which regulates the expression of multiple genomic loci by modulating higher-order chromatin structure.[21] Down-regulation of the gene in an aggressive metastatic breast cancer cell line restored cell polarity *in vitro* while inhibiting tumour growth and metastasis in mice. Furthermore, immunohistochemical analysis of a large cohort of breast tumour samples found SATB1 expression to be an independent prognostic factor for breast cancer metastasis.[21] Also emerging as key regulators of the metastatic process are miRNAs, which are a class of small, non-coding RNA molecules that have the ability to co-ordinate global changes in gene

expression by inhibiting the translation of multiple mRNA transcripts via the RNAi pathway.[22] They have been shown to play a key role in numerous physiological processes from embryogenesis to tumourigenesis, and now metastasis. A recent study identified miRNA-335 as a potent metastasis suppressor whose expression was lost in both metastatic cell lines and tumours, resulting in a dramatic increase in metastases through up-regulation of the transcription factor SOX4, which directs the expression of numerous genes implicated in tumour progression and metastasis.[23]

Managing metastatic breast cancer

Compared with early-stage breast cancer, there are few proven standards of care for the management of MBC. However, current clinical recommendations from the European Society of Medical Oncology (ESMO) advocate the use of an interdisciplinary team of healthcare professionals to provide patients with personalised psychosocial, supportive and symptom-related interventions.[24] A brief overview of the different treatment strategies for MBC is provided in **Table 2**. Patients with resistant or unresponsive disease may be considered for inclusion in appropriate clinical trials, which in Ireland are largely co-ordinated by the Irish Clinical Oncology Research Group (ICORG).

One of the main challenges with respect to metastases is the fact that most are discovered at such an advanced stage in their development that they are often incurable. However, new techniques are emerging, which have the ability to detect disseminating tumour cells present at the single-cell level in bone marrow and peripheral blood.[25] Although much success has been achieved with the detection of disseminating tumour cells in bone marrow,[26] efforts are now being directed towards the detection of circulating tumour cells (CTCs) in peripheral blood. A novel system called CellSearch™(Veridex), which detects CTCs via immunocytochemistry, has just been approved by the US Food and Drug Administration for use in the management of advanced breast cancer patients.[27]

Conclusion

Metastasis is the major contributor to mortality in patients with breast cancer, and most other malignancies. However, in comparison with tumourigenesis, relatively little is known about the molecular mechanisms or determinants of this complex process. Consequently, early stage breast cancer is typically managed very successfully, while metastasis remains a significant challenge. Although research in the field is very active, it will take some time before these hypotheses are integrated to yield a definitive model of breast cancer progression and metastasis. These studies are crucial as they provide an insight into the molecular basis of the disease process. This is not only important as a means of identifying novel points for therapeutic intervention, but for the discovery of new biomarkers that could be used to determine prognosis or response to therapy.

Table 2: Overview of current ESMO clinical recommendations for treatment of main metastatic breast cancer sub-types: luminal-type (hormone receptor-positive); HER2/neu positive breast cancer; and, basal-type (hormone receptor-negative).[24]

Luminal-type breast cancer (hormone receptor-positive)

- Endocrine therapy is the preferred option except if clinically aggressive disease mandates a quicker response or if resistance is suspected.
- The choice of endocrine agent should be individualised according to the patient's safety profile, co-morbidities and tumour biology.
- Tamoxifen with ovarian ablation is typically recommended for premenopausal patients, while third-generation aromatase inhibitors are advised for postmenopausal patients.

HER2/neu-positive breast cancer

- Patients should be treated with trastuzumab (Herceptin) with/without chemotherapy, and cardiac monitoring should be performed before/after commencement to monitor for cardiotoxicity.
- Lapatinib (Tyverb/Tykerb) may also be used and has shown a significant increase in time to progression in combination with capecitabine (Xeloda) in patients progressing after trastuzumab (Herceptin) treatment.
- Other anti-HER2/neu agents such as pertuzumab (Omnitarg) are currently under investigation.
- Combinations of trastuzumab (Herceptin) with other biological agents with/without chemotherapy to tackle the problem of resistance to trastuzumab (Herceptin) are currently under investigation.

Basal-type breast cancer (hormone receptor-negative)

- Patients with hormone receptor-negative tumours are candidates for cytotoxic chemotherapy, of which taxane-based regimens are the only ones where level one evidence is available.
- In the absence of the need for a rapid and significant response for symptom control or life-threatening disease, preference is given to the sequential use of a single cytotoxic agent, as it is associated with reduced toxicity and improved quality of life in comparison with combination chemotherapy.
- The duration and number of regimens should be tailored to each individual patient; however, high-dose chemotherapy should not be proposed.

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