

# Specific cell differentiation in breast cancer: a basis for histological classification

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Received 14 February 2021

Accepted 20 February 2021

Published Online First

28 July 2021

## ABSTRACT

Breast parenchyma progenitor cells show a high degree of phenotypic plasticity reflected in the wide range of morphology observed in benign and malignant breast tumours. Although there is evidence suggesting that all breast cancer (BC) arises from a common epithelial progenitor or stem cell located at the terminal duct lobular units (TDLUs), BC shows a broad spectrum of morphology with extensive variation in histological type and grade. This is related to the complexity of BC carcinogenesis including initial genetic changes in the cell of origin, subsequent genetic and epigenetic alterations and reprogramming that occur at various stages of BC development and the interplay with the surrounding microenvironment, factors which influence the process of differentiation. Differentiation in BC determines the morphology, which can be measured using histological grade and tumour type. Histological grade, which measures the similarity to the TDLUs, reflects the degree of differentiation whereas tumour type reflects the type of differentiation. Understanding BC phenotypic differentiation facilitates the accurate diagnosis and histological classification of BC with corresponding clinical implications in terms of disease behaviour, prognosis and management plans. In this review, we highlight the potential pathways that BC stem cells follow resulting in the development of different histological types of BC and how knowledge of these pathways impacts our ability to classify BC in diagnostic practice. We also discuss the role of cellular differentiation in producing metaplastic and neuroendocrine carcinomas of the breast and how the latter differ from their counterparts in other organs, with emphasis on clinical relevance.

## INTRODUCTION

Breast cancer (BC) is currently considered a heterogeneous group of diseases with a wide range of morphological appearances. Controversy persists as to whether the phenotypic diversity of BC is related to a well-programmed histogenesis pathway at an early stage of cancer development or specific subsequent genetic events at various stages of carcinogenesis (differentiation) regardless of the cell of origin.<sup>1</sup> In the early BC classification, the use of the term ductal perpetuated the traditional concept that these tumours derived from mammary ductal epithelium whereas lobular carcinomas were deemed to have arisen from lobules. Subsequent studies<sup>1,2</sup> demonstrated that both types of carcinoma most commonly develop in terminal duct-lobular units (TDLUs) and that lobular carcinoma occurs due to loss of E-cadherin gene function at

an early stage of carcinogenesis. This constituted a paradigm shift at that time and was the beginning of the end of the histogenetic implications of the ductal and lobular terminology.

Although mammary gland originates from ectoderm and mesoderm during embryogenesis that form the breast parenchyma and stroma, respectively, most BC subtypes are now considered to arise from BC progenitor or stem cells<sup>2-4</sup> that are likely to be region specific and multipotent.<sup>3</sup> These multipotent cells can give rise to various tissue types and hence multiple tumour morphologies. Although these stem cells are frequently located in the TDLU, they can be located in ducts.<sup>3</sup> This is supported by finding carcinomas such as encapsulated and solid papillary carcinoma predominantly located in large ducts without the involvement of TDLUs. Ductal carcinoma in situ (DCIS) also most commonly affects medium-sized ducts with less frequent involvement of lobules (cancerisation of lobules),<sup>5</sup> the latter possibly representing spread from involved ducts rather than de novo change within lobules. Although male breast lacks TDLUs, males also develop BC with or without gynaecomastia. Therefore, current evidence suggests that BC arises from cancer stem cells located more frequently, but not exclusively, in the regions of the TDLUs.

Gene expression profile studies have categorised BC into distinct molecular classes in line with the two main cell lineages in the breast, which are luminal and myoepithelial/basal. The other main category, the HER2 positive BC group, reflects an oncogenic event rather than cell lineage or cell of origin.<sup>6</sup> There is also evidence that most basal tumours derive from luminal progenitors rather than from basal stem cells with subsequent acquisition of basal-like genotypic and phenotypic characteristics. Deletion of *BRCA1* in mouse mammary epithelial luminal progenitor cells produced tumours that phenotypically resemble human *BRCA1* associated BC while directing *BRCA1* deficiency to basal cells generated tumours that did not histologically resemble basal tumours despite having the molecular profile of basal cells.<sup>7</sup> Forced expression of *HRAS* (Q61R) in non-malignant ER-negative breast epithelial cells with or without a *PIK3CA* (H1047R) somatic knock-in resulted in the acquisition of the myoepithelial cell phenotype with expression of myoepithelial cell markers, a reduction in E-cadherin expression and an increase in AKT signalling.<sup>8</sup> These results suggest that tumour phenotypes may not directly reflect histogenesis.

Some rare BCs with characteristic morphology and behaviour show specific molecular alterations



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**To cite:** Rakha E, Toss M, Quinn C. *J Clin Pathol* 2022;**75**:76–84.

that may explain their distinct phenotypes such as translocation and formation of specific fusion genes in secretory carcinoma, adenoid cystic and mucoepidermoid carcinomas and the *IDH2* gene mutation in solid papillary carcinoma with reversed polarity.<sup>9</sup> Our recent study of the morpho-molecular correlates of BC<sup>10</sup> identified specific sets of differentially expressed genes associated with various morphological features characteristic of differentiation. It demonstrated that morphology reflects the underlying molecular profiles and vice versa. These observations have reinforced the perception that cell of origin, differentiation and genotype–phenotype correlation in BC is a complex process. Deciphering such a complex process can improve our understanding of BC development, biology and behaviour. This will help evaluate the likely biological significance of these morphological types and provide a potential proper approach for their management.

Differentiation in BC can be measured morphologically using histological grade and tumour type. Histological grade, which measures the similarity of the tumours to the TDLUs, reflects the

degree of differentiation whereas tumour type reflects the type of differentiation. In previous studies, we discussed the role of histological grade as a reflection of the degree of differentiation and the molecular profile in BC.<sup>10 11</sup> In this review, we discuss the concept of BC differentiation and its impact on tumour histological type classification, emphasising potential management implications.

### BC differentiation

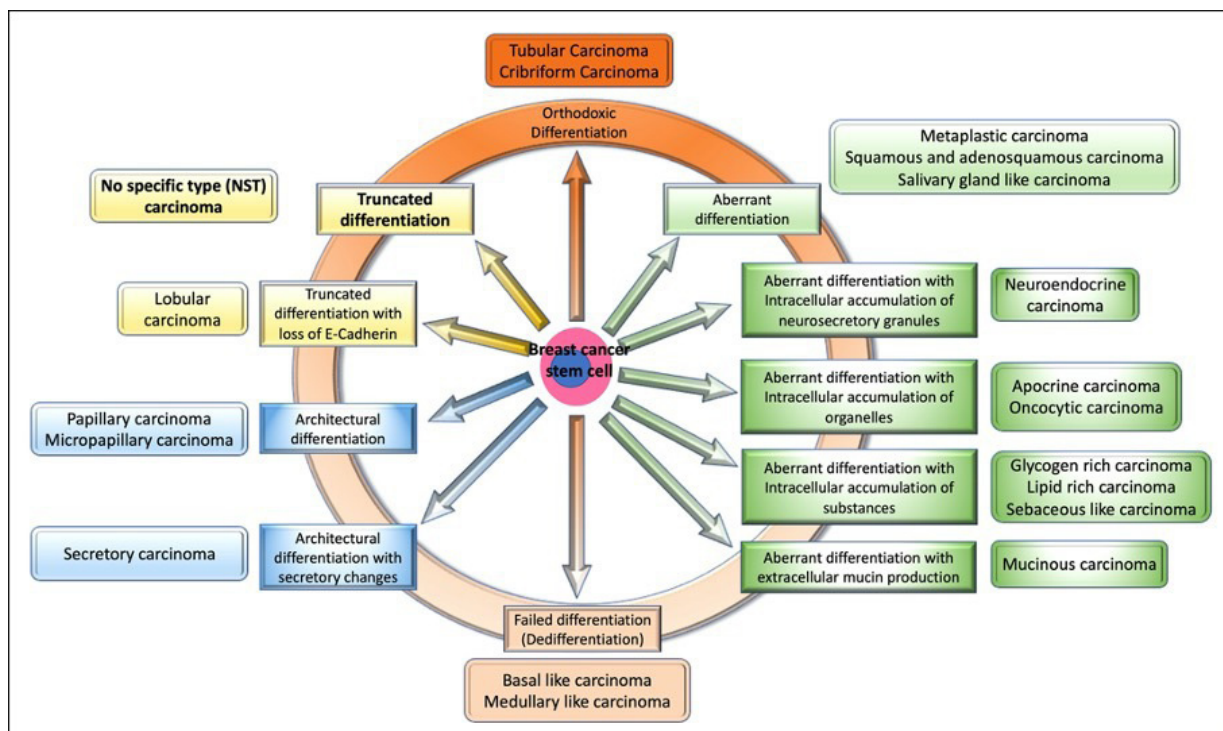
In biology, the term differentiation denotes a process by which proliferating cells gradually acquire a more specialised function by changing phenotype. Although the terms differentiation, histogenesis and morphogenesis are used interchangeably, they are different. Table 1 summarises the definitions of these terms alongside other terms used to describe cellular phenotypic changes. In normal development, differentiation is unidirectional, termed forward or lineage differentiation. The complex process of cellular proliferation is co-ordinated by master

**Table 1** Definitions of related terminologies

Terms	Definition	Examples
Carcinogenesis (oncogenesis/tumorigenesis)	It is the formation of a cancer, in which normal cells (cancer stem cell) are transformed into malignant cells regardless of the type of cells. The process is characterised by changes at the genetic and epigenetic levels. Once cancer cells are produced, they undergo a process of natural selection and the cells with new genetic changes that enhance their survival multiply faster and dominate the growing tumour. Late in the carcinogenesis, cancer cells often acquire additional genetic or epigenetic changes that may alter their phenotype and behaviour.	BC arise from breast epithelial cancer stem cells whereas breast sarcoma arises mesenchymal type stem cells.
Histogenesis	Histogenesis in cancer refers to the kind of normal cell from which the cancer may arise. Tumours are classified as epithelial, mesenchymal, neuroendocrine or lymphoid depending on their cell of origin. Most tumours show the histopathological features of their normal cellular counterpart and can be classified accordingly. Therefore, breast carcinomas arise from a specific (progenitor or stem) breast epithelial cells and show similar features.	Cell of origin of the BC
Differentiation	Cellular differentiation is the process in which a cell changes from one cell type to a more specialised cell type. Differentiation in cancer describes how close the tumour resembles the tissues and cells of origin; in the breast, it is TDLUs. Tubule formation requires differentiated polarised neoplastic cells that orientate in relation to adjacent cells, adhere together and distinguish between the apical and basal surface. Such ability to form tubules is indicative of the tumour differentiation. Differentiation significantly changes the cell's size, shape, metabolic activity and function. It is mainly due to modifications in gene expression rather than a change in the DNA sequence. It can be examined by evaluation of the cell function, epigenome, transcriptome and proteome profiles.	Epithelial cancer stem cells differentiate to luminal or myoepithelial end differentiated cells.* In revertant DCIS at metastatic sites. More tubule formation and lower grade at metastatic sites
Metaplasia	It is the transformation of one differentiated cell type to another differentiated cell type, which may be part of a normal maturation process, or caused by an abnormal stimulus	Apocrine, squamous, and mucinous metaplasia
Trans-differentiation	It means transforming one mature somatic cell type into another mature somatic cell. It is a type of metaplasia; Also known as 'lineage reprogramming'.	MBC; squamous, spindle cell and matrix producing, apocrine carcinomas
Dedifferentiation	It is a cellular process in which a partially or terminally differentiated cell reverts to cells with characteristics of an earlier developmental stage. Cells can lose properties they originally had, such as protein expression or change shape.	Basal like carcinoma.
Cell potency	It refers to the ability of stem cells to differentiate into specialised cell types. Cells with the greatest potency can generate more cell types than those with lower potency. The somatic stem/progenitor cells can be classified according to cell potency into: <ul style="list-style-type: none"> <li>▶ Pluripotent stem cells, which can give rise to all cell types of the body (somatic or germ cells).</li> <li>▶ Multipotent stem cells, which can develop into a limited number of cell types in a particular lineage (somatic but not germ).</li> <li>▶ Unipotent stem cells, which can differentiate into a single "target" cell type only.</li> <li>▶ Progenitor cells, which are, like the unipotent stem cell, capable to differentiate into a specific type of cell. However, progenitor cells, unlike stem cells can divide only a limited number of times. Progenitor cells are in the centre between stem cells and fully differentiated cells.</li> </ul>	Breast normal epithelial stem or progenitor cells and BC stem cells
Epithelial mesenchymal transition (EMT)	EMT constitutes the loss of hallmark structures and physiologic properties associated with the epithelial cells and the gain of new properties, including migratory and invasive growth patterns (transdifferentiation of epithelial cells to a mesenchymal phenotype). EMT is observed in <i>in vitro</i> models. <i>In vivo</i> , it can be linked to tumours showing mesenchymal spindle cell differentiation. Lobular carcinoma shows loss of E-cadherin but not part of EMT.	Spindle cell MBC

\*The terminal differentiation in which the differentiated cell permanently leaves the cell cycle and often expresses a range of genes characteristic of the cell's final function is not seen in the breast, and normal, benign and malignant BC cells do not permanently leave the cell cycle.

BC, breast cancer; DCIS, Ductal carcinoma in situ; MBC, metaplastic breast carcinoma; TDLUs, terminal duct lobular units.



**Figure 1** The various differentiation pathways that leads to the difference in the morphology of the tumours as recognised histological tumour types.

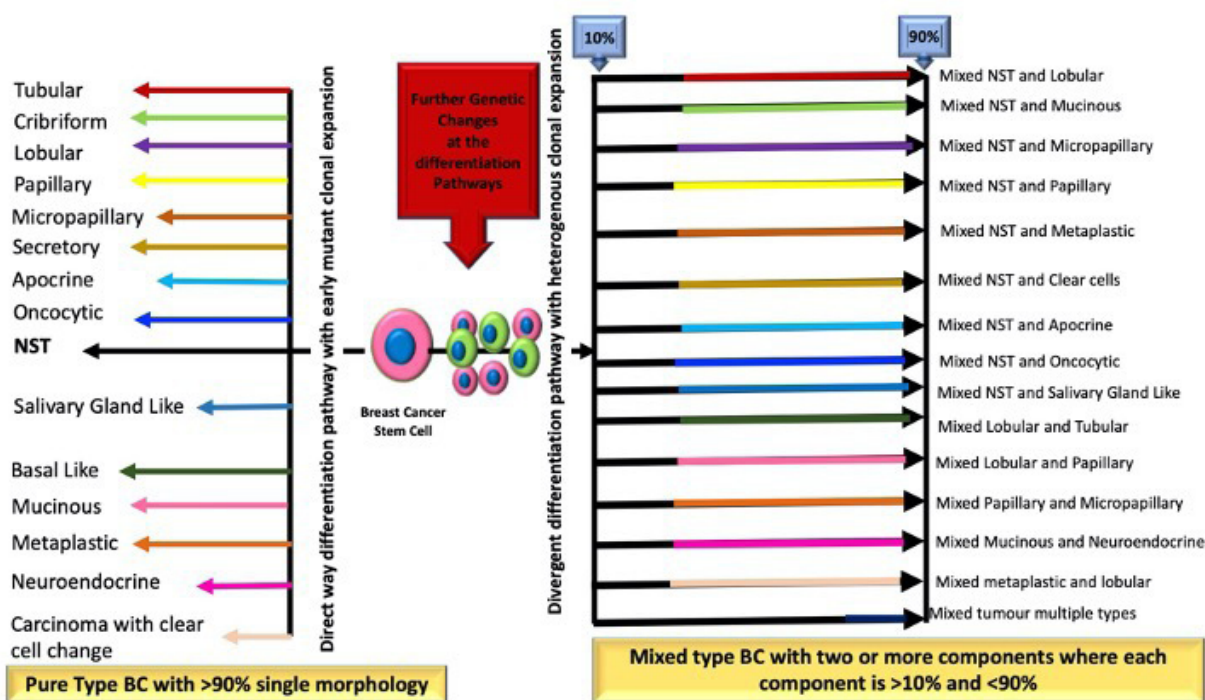
transcription factors that drive forward differentiation.<sup>12 13</sup> In the context of BC, forward (orthodox) differentiation means that the proliferating BC stem progenitor cells differentiate towards end differentiated luminal epithelial cells (lineage), with the formation of tubules and glands as seen in tubular carcinoma and low-grade invasive breast carcinoma of no special type (IBC-NST). Forward differentiation can also occur at a later stage of carcinogenesis as evidenced by DCIS like structures in metastatic disease (revertant DCIS).<sup>14</sup> In BC, this lineage (luminal epithelial cell) specific differentiation is frequently impeded, altered or reversed (figure 1), the causes of which are mostly unknown.<sup>12 13 15</sup> The proliferating malignant cells may show one of the following:

1. Failed or backward differentiation (dedifferentiation) of the luminal epithelial cell lineage. In this situation, BC shows dominant primitive stem cell features or less differentiated/undifferentiated carcinoma appearances. Differentiation failure typically reflects aggressive tumour transformation, usually resulting in high-grade BC, for example, basal-like. The majority of the oestrogen receptor (ER) negative BC NSTs display features that reflect failed differentiation and are characterised by aggressive clinical behaviour.
2. Aberrant differentiation towards other cell lineages (trans-differentiation), for example, myoepithelial cell (basal-like), apocrine cell, squamous cell, salivary gland or skin adnexal like structures. Reprogramming of malignant epithelial cells towards mesenchymal cells, following an oncogenic insult, resulting in the development of metaplastic/mesenchymal-like carcinomas is an example of aberrant differentiation. This involves epithelial mesenchymal transition with gradual loss of the epithelial phenotype and acquisition of a mesenchymal phenotype<sup>15</sup> that may feature spindle cell, chondroid, osteoid or rhabdoid differentiation.
3. Acquire a specific type of differentiation resulting in the acquisition of a new phenotype of the malignant cells. This

includes: (1) accumulation of intracellular materials such as mucin, lipid, glycogen, neurosecretory granules or melanin, (2) accumulation of specific organelles, for example, mitochondria as in oncocytic carcinoma and ribosomes such as apocrine carcinoma, (3) excessive secretion of extracellular substances as in mucinous carcinoma, (4) acquire specific architecture not characteristic of normal breast TDLUs as seen in papillary and micropapillary carcinomas or (5) accumulation or excessive secretion of material combined with a particular architecture as seen in mucinous cystadenocarcinoma or micropapillary mucinous carcinoma.

4. Divergent differentiation in which BC derived from a single stem cell undergoes divergent differentiation early in the evolution of the tumour resulting in biphasic tumours, for example, adenocarcinoma cells coexisting with squamous or spindle cells or a combination of epithelial-like and myoepithelial like cells.<sup>16</sup> Divergent differentiation is likely to result in multiple tumour components with different morphology as seen in some examples of metaplastic BC (MBC). It can also explain mixed tumours in which an IBC-NST clone loses E-cadherin function early in evolution and differentiate to lobular carcinoma resulting in mixed IBC-NST and lobular carcinoma (figure 2).

Most of the genetic alterations that occur during BC carcinogenesis, resulting in such heterogeneous morphology, are not well defined. However, there are well known specific genetic alterations that can affect tumour morphology. Loss of E-cadherin function produces the dyscohesive cell arrangement typical of invasive lobular carcinoma in a tumour which otherwise has identical molecular alterations to low-grade IBC-NST and tubular carcinoma. Mutations of the *IDH2* gene are associated with breast solid papillary carcinoma with reversed polarity<sup>17 18</sup> whereas chromosomal translocations resulting in the development of specific fusion genes can lead to unique morphology in a small proportion of BC. Examples include mucoepidermoid



**Figure 2** The mixed lines of differentiation with two main categories identified. On the right side of the figure, a single differentiation pathway predominates or represents the only pathway resulting in a pure tumour type which shows specific tumour characteristics in 100% of the tumour with or without another minor component in less than 10% of the tumour. In the left side of the figure, more than one differentiation pathways are active. The tumours may show two or more components, but none of the differentiation pathways is predominant enough to produce more than 90% of the tumour. The other pathways are also active, so they produce more than 10% of the tumour. BC, breast cancer; NST, no special type.

(*CRTC3-MAML2* fusion gene), secretory (*ETV6-NTRK3* fusion gene) and adenoid cystic carcinomas (*MYB-NFIB* fusion gene).

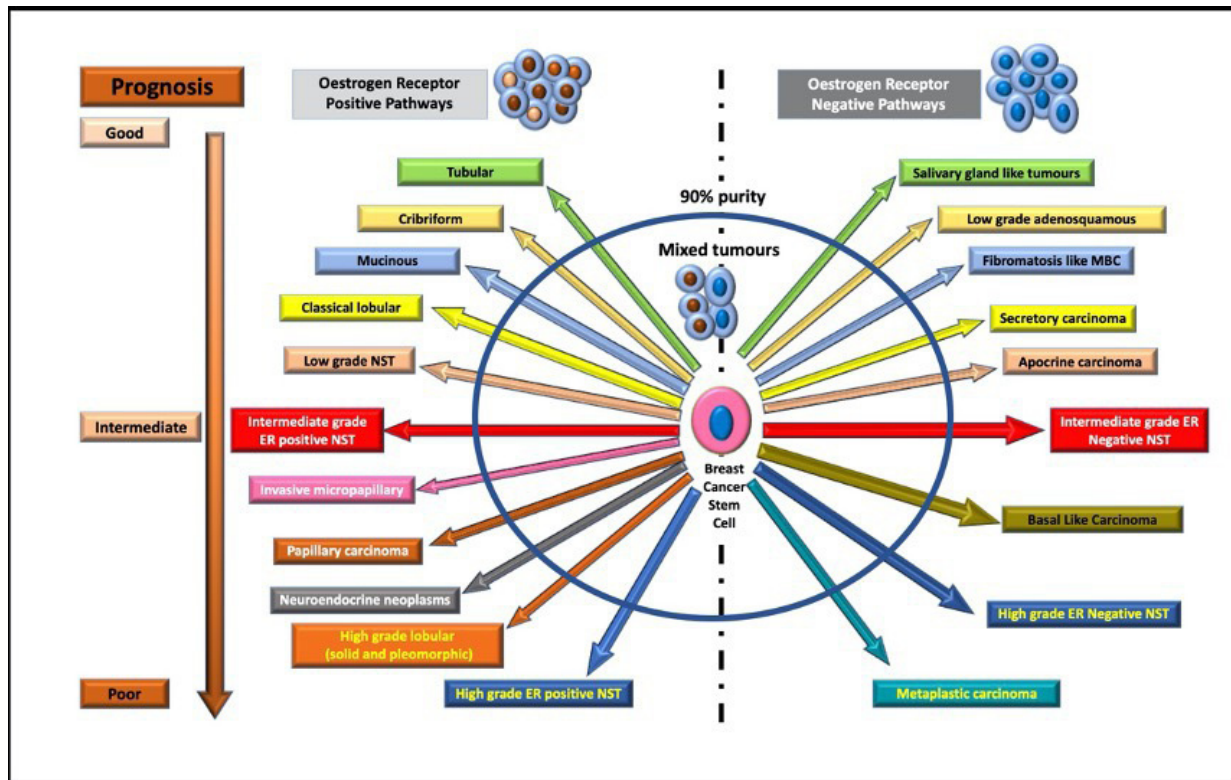
ER and HER2 are the main determinants of BC molecular profile, clinical behaviour and response to therapy. However, the relationship between ER and HER2 expression and BC differentiation (type and grade) appears to represent a complex process that is poorly understood. IBC-NST BCs, for example, may show identical morphology with very different ER and HER2 biomarker profiles. Between 10% and 40% of metastatic BCs show a change in ER status without a change of tumour grade or type.<sup>19</sup> Although ER is correlated with BC histological grade and some specific tumour types (figure 3), ER positive BC comprises a spectrum of disease with a variety of tumour types and grade and not all ER negative tumours are poorly differentiated. Some ER negative tumours such as secretory and adenoid cystic carcinoma are low grade and indolent with a better prognosis compared with some of the well-differentiated ER positive BC types. HER2, overexpressed in 15% of BC, is uncommonly seen in the well-differentiated tumours and in certain tumour types including classical lobular, medullary-like, metaplastic and salivary gland-like tumours. It is unclear whether HER2 drives differentiation in some BCs or has a limited interaction with the tumour, mainly promoting tumour growth and proliferation. Although the precise relationship between these two key biomarkers and BC differentiation has not been clarified, knowledge of the correlation between histological grade, morphological type, and receptor status is useful in diagnostic practice.

Proliferating BC cells may pursue one line of differentiation producing uniform morphology that can be recognised morphologically as a pure or special histological subtype; when the feature is seen >90% of the tumour. However, differentiation in BC is generally a complex and dynamic process governed by

multiple inter-related factors with varying impact depending on the stage of the carcinogenesis process from initiation to the development of distant metastases. Most BCs are the product of more than one line of differentiation with resultant heterogeneity (both intratumoural and intertumoural) at morphological and molecular level.<sup>20</sup> Intratumoural heterogeneity is the basis for mixed tumours that usually comprise a combination of IBC-NST and a special subtype, the latter accounting for at least 10% of the tumour.<sup>21</sup> Intertumoural heterogeneity allows the classification of BC into different subtypes that likely represent biologically distinct disease entities with variable presentation, morphology, clinical behaviour and response to therapy.

### Differentiation and BC histological types

Based on morphology, invasive BC is currently subclassified according to growth pattern and degree of differentiation, reflecting how closely a tumour resembles normal breast TDLUs.<sup>21</sup> This is achieved by assessment of histological type and tumour grade. BC grade, which is measured by the Nottingham grading system (NGS), refers to the semiquantitative evaluation of three important biology dependent morphological features: (1) degree of tubule or gland formation, (2) nuclear pleomorphism and (3) mitotic count.<sup>22</sup> NGS applies to all BC histological subtypes. However, not BCs of similar grade represent the outcome of the same line of differentiation, for example, tubular and invasive cribriform carcinoma are grade 1 by definition, whereas IBC with medullary features and basal-like BC are high-grade tumours. Moreover, most BC histological types such as NST, lobular, mucinous and metaplastic carcinomas show varying histological grade while maintaining specific tumour type characteristics. Therefore, both histological grade and



**Figure 3** The relationship between the various differentiation pathways related tumour types and oestrogen receptor (ER) expression and prognosis with some tumours are typically ER positive and some are typically ER negative and each is associated with variable prognosis. MBC, metaplastic breast cancer; NST, no special type.

tumour type (as the morphological reflection of differentiation) have intrinsic prognostic significance that can be maximised by combining both.<sup>22</sup>

The fifth edition (2019) WHO Classification of Breast Tumours continues to recognise several special types of BC, which together account for up to 25% of all invasive BCs.<sup>21</sup> Knowledge of these special types helps pathologists to recognise that a tumour is of primary breast origin and may provide clinically relevant information. For example, a diagnosis of invasive lobular carcinoma on needle core biopsy usually leads to further preoperative imaging due to the increased incidence of multifocality and bilaterality. Invasive lobular carcinoma is also less likely to respond to chemotherapy which is important in patient selection for neoadjuvant chemotherapy. Metaplastic carcinoma is generally associated with a poor prognosis and a limited response to chemotherapy.

The fifth edition WHO working group<sup>21</sup> also introduced some changes concerning tumour typing, reflecting not only improved understanding of the tumour biology including molecular alterations and clinical behaviour but also diagnostic concordance and level of evidence for their designation as special tumour types. Salivary gland-like tumours, apocrine carcinoma and invasive papillary carcinoma continue to be recognised as special types, despite their rarity, as they have distinct molecular, morphological and clinical features. Some rare tumours for example, tall cell carcinoma of breast with reversed polarity and mucinous cystadenocarcinoma are now also recognised as special type BCs. However, other tumour types, considered to represent end of differentiation of IBC-NST, have been reassigned to IBC-NST category with a designation of special morphological pattern. Perhaps the most important changes involve medullary/medullary-like carcinomas.

Medullary/medullary like carcinoma is now categorised as a special morphological pattern of the IBC-NST category with recognition of prominent tumour infiltrating lymphocytes (TILs) rather than as a separate special type BC. The diagnosis of medullary carcinoma was originally based on a particular constellation of tumour characteristics, each of which may be seen in IBC-NST, which led to low diagnostic concordance among pathologists. The superior prognosis reported for medullary/medullary-like carcinoma compared with grade and stage matched IBC-NST is now seen as a reflection of prominent stromal TILs rather than the syncytial growth pattern or absence of DCIS.<sup>23</sup> In view of the limited clinical relevance, the overlap with the aggressive basal-like and *BRCA1* tumours and the low concordance rates in diagnostic practice, these tumours are regarded as part of the spectrum of IBC-NST differentiation with a TILs rich stroma.

Other tumours recategorised as variants of IBC-NST with special morphological patterns include the so-called glycogen rich, lipid-rich, sebaceous and oncocytic carcinomas. These tumours are currently considered as IBC-NST with a spectrum differentiation that features intracellular accumulation of various constituents. The prognosis of these tumours does not differ from grade matched classical IBC-NST and criteria for diagnosis were inconsistent with the modern approach to categorisation of special type BC. For example, the cut-off for sebaceous differentiation for a diagnosis of sebaceous carcinoma was reported to be 50%,<sup>24</sup> and most cases described in the literature are not morphologically identical to conventional type sebaceous carcinomas of the skin and eyelid. Similarly, oncocytic and glycogen rich carcinomas are not as morphologically distinct as oncocytic carcinoma or clear cell renal cell carcinoma, respectively. The clinical relevance of such entities is diagnostic and awareness of their possible occurrence as primary breast

tumours with exclusion of metastases is important in clinical practice.

### BC differentiation and metaplastic carcinoma

Although MBC is rare comprising 0.3%–1.5% of BC, it constitutes a heterogeneous group of tumours with multiple subtypes reflecting variable differentiation pathways, histological appearances and clinical behaviour.<sup>25–30</sup> Two main pathways are recognised; squamous and mesenchymal differentiation, the latter including spindle cell and matrix producing differentiation. MBC with squamous differentiation includes the indolent low-grade adenosquamous carcinoma and the aggressive high-grade adenosquamous carcinoma. Similarly, spindle cell MBC includes the excellent prognosis ‘fibromatosis-like’ MBC and the very aggressive high-grade spindle cell MBC.<sup>1–31</sup> Heterologous matrix-producing transdifferentiation of mammary epithelial/myoepithelial cells may be seen in benign and malignant lesions.<sup>32–34</sup> Matrix producing BC is characterised by malignant osteoid or cartilaginous tissue.

Primary squamous cell MBC is derived from mammary BC stem cells with differentiation towards squamous cells. Although it is morphologically similar to squamous cell carcinoma of the breast skin, breast squamous cell carcinoma is histogenetically distinct from the cutaneous counterpart, which is derived from epidermal cells. In practice, the distinction of these tumours is essential due to significantly different management implications. Helpful features in reaching a diagnosis of squamous cell MBC include the tumour location within the breast, the presence of DCIS, IBC-NST components, other breast MBC components, particularly spindle cells. Cytokeratin 7 positivity may also assist the diagnosis.

In ‘mesenchymal-looking’ BC, the diagnosis of MBC is based on evidence of carcinomatous differentiation as demonstrated by the presence of a conventional-type invasive breast adenocarcinomatous or squamous-cell component and/or a DCIS. In the absence of these features, the diagnosis of MBC requires the demonstration of epithelial differentiation using immunohistochemistry (ie, CKs expression). In rare instances, evidence of a carcinomatous phenotype is lacking, and the entire tumour comprises mesenchymal-looking spindle-cell or a matrix producing sarcomatous component. In the absence of features of phyllodes tumour<sup>33</sup> or evidence of metastatic sarcoma, melanoma or lymphoma, these lesions were frequently categorised as primary breast sarcoma including osteosarcoma, chondrosarcoma or primary breast sarcoma of NST.<sup>35–39</sup> In previous studies,<sup>40,41</sup> we provided evidence that these tumours are likely to represent the extreme end of trans-differentiation of BC towards mesenchymal-type spindle cell or matrix producing tissue with loss of morphological and immunohistochemical expression of their true carcinomatous nature.<sup>1–40</sup> This was based on the following observations: (1) not all well-established mesenchymal-looking MBCs contain DCIS, squamous or adenocarcinomatous components, (2) DCIS is absent in a proportion of conventional well-established IBC, (3) primary breast sarcoma is extremely rare and constitutes a diagnosis of exclusion,<sup>36–42–44</sup> and a proportion of tumours classified as breast sarcoma have been re-classified as MBC following detailed CK IHC work-up<sup>36–45</sup> and (4) BCs variably lack expression of one or more CKs, a phenomenon that is more commonly observed in MBC,<sup>1–46–52</sup> or may show only focal expression, suggesting that the existence of MBC with loss of CK expression should be accepted.<sup>41–42–46–47–52–55</sup> In our view, the presence or absence of CK expression should not be considered irrefutable evidence to support tumour histogenesis

and categorisation as carcinoma or sarcoma, respectively. Rather loss of CK expression reflects extreme differentiation of BC to a mesenchymal phenotype. Similarly, a sarcoma derived from mesenchymal cells may acquire CK expression as a reflection of carcinomatous differentiation, as seen in some malignant phyllodes.<sup>56</sup> We advocate that breast tumours with ‘mesenchymal’ morphology can be managed as poorly differentiated MBCs provided that other tumours included in the differential diagnosis are excluded.<sup>41</sup>

Conventional IBC-NST carcinoma occasionally contains minor components of metaplastic elements with squamous and/or mesenchymal appearances.<sup>57</sup> However, when the metaplastic components form a significant proportion of the tumour, the term MBC is used.<sup>21</sup> The percentage of metaplastic elements required to make the diagnosis varies widely in the literature (>10%, ≥20%<sup>58</sup> and ≥50%<sup>28</sup>) and no cut-off was used in the recent WHO book.<sup>21</sup> This reflects the difficulty in many of these tumours in distinguishing the metaplastic from the non-metaplastic adenocarcinomatous components. In some MBC variants the carcinomatous component is an integral rather than a coexistent element of the tumour for example, adenosquamous carcinomas in contrast to spindle cell and matrix producing MBC. In many mixed tumours, the adenocarcinoma component shares features with the metaplastic component such as high histological grade and/or triple negative phenotype. In practice, we observe two main types of MBC: (1) tumours with metaplastic and adenocarcinomatous components with morphological and/or biomarker overlap, considered as MBC regardless of the percentage of the tumour occupied by metaplastic elements, and (2) tumours with a distinct metaplastic component (eg, spindle cell or matrix producing) that may coexist with another special type or IBC-NST component. These tumours are regarded as pure MBC if the metaplastic component exceeds 90%, and as mixed tumours if the metaplastic component accounts for >10% and <90% of the tumour. In general, the presence of a metaplastic element is associated with aggressive clinical behaviour and is likely to drive the behaviour of the tumour regardless of percentage.

### Differentiation and the difference between breast neuroendocrine neoplasms and the conventional neuroendocrine neoplasms of other organs

Neuroendocrine (NE) differentiation in BC was first described in mucinous carcinoma,<sup>59</sup> as an invasive carcinoma morphologically similar to intestinal carcinoid based on positive silver staining. Since their description, NE tumours of the breast have been a source of confusion regarding origin, terminology, diagnostic criteria, the lack of distinction between invasive and in situ lesions and management implications. Breast NE neoplasms (NENs) are currently described as tumours with morphology similar to gastrointestinal and pulmonary NE tumours and expression of one or more NE markers (specifically chromogranin and synaptophysin). However, there are a number of key differences between NENs of the breast and these organs. The classical organoid features of carcinoid tumours of the lung and gastrointestinal tract (regular nests, insular pattern, ribbons, cords and rosettes) are not typical features of primary breast NENs. Clinical syndromes related to hormone production are extremely rare in breast NENs. In contrast with lung, gastrointestinal tract, pancreas and prostate, in which NE cells are normal constituents and explain the presence of NENs in these organs,<sup>60</sup> the existence of normal NE cells or benign NE tumours in the breast has not been confirmed.<sup>61</sup> NE differentiation is recognised

to occur at relatively high frequency in conventional IBC-NST and some special type BCs and most breast NENs are likely to represent mixed tumours with a component of conventional type in situ or invasive carcinoma.<sup>62</sup> For these reasons the existence of NENs in the breast is believed to relate to NE pathway differentiation of cancer stem/progenitor 'epithelial' cells rather than true histogenesis from NE cells; they are likely tumours of epithelial origin. Further refinement of the classification of breast NENs that consider the differentiation of these tumours is needed to improve their diagnostic reproducibility and consolidate the clinical significance of each diagnosis.

Apart from rare cases of small cell carcinoma, analogous to the pulmonary counterpart, that has distinct morphology and clinical behaviour, the definition of NENs in the breast is widely variable, resulting in differences in reported incidence according to the different diagnostic criteria applied (from 0.1% to 18%<sup>63</sup>). The terms 'NE tumour' and 'NE carcinoma', utilised for NENS at other organ sites, are confusing and should be avoided as almost all NENs are considered to represent invasive adenocarcinomas. The distinction between a diagnosis of large cell NE carcinoma and a grade 3 IBC with NE differentiation is not clear and may refer to the same breast tumour.

The main aims of the classification of breast NENs in practice relate to their identification as primary BCs, exclusion of metastatic NENs from other sites and recognition to enable future studies that may show clinical relevance. In this article, we acknowledge the WHO classification of NENs of the breast<sup>21</sup> that aimed to apply a uniform classification framework for all NENs in different organs including the breast; however, we currently believe that such a classification system needs modification to avoid confusion in clinical practice and to recognise the unique nature of breast NENs. In line with the WHO approach, specific breast tumours, including hypercellular type B mucinous carcinoma and invasive SPC that show extensive NE differentiation and are associated with a superior prognosis, should be recognised separately as distinct special type BCs. These tumours should not be designated as NETs to avoid confusion by using different names for the same tumours.

The designation of small cell NE BC (SCNEBC) as a special tumour type should remain unchanged as it is a unique and well-recognised tumour that can be identified based on morphology, with sufficient evidence of characteristic and predictable clinical behaviour. SCNEBC should be classified as such to avoid confusion with other grade 3 tumours with NE differentiation not showing the small cell phenotype. In line with the WHO classification,<sup>21</sup> tumours showing two distinct components with SCNEBC comprising 10%–90% of the tumour, should be designated as mixed carcinomas (eg, NST or other special type and small cell carcinoma) and the SCNEBC percentage should be reported (eg, 'Mixed IBC-NST and SCNEBC (50%)').

IBC-NST tumours with extensive NE differentiation (>90%), evident morphologically and on IHC, should be considered a special pattern of IBC-NST representing the end of the spectrum of the NE differentiation pathway (IBC-NST with NE pattern). There is no consistent evidence of prognostic significance or unique clinical behaviour associated with this degree of NE differentiation to support their classification as a special type of BC and these tumours are usually managed as for conventional IBC-NSTs. This differs from NENs of other organs such as lung and gastrointestinal tract, where precise identification and classification of these tumours is mandatory for appropriate management. Like other types of IBC, these tumours are graded using the NGS and not according to that used to grade NENs in other organs.<sup>22</sup>

## Tumour differentiation and BC heterogeneity

Several recent studies of BC have documented not only inter-tumoural but also intratumoural molecular and genetic heterogeneity.<sup>64–70</sup> The clonal divergence between primary carcinomas and corresponding metastases has been demonstrated, and there is evidence to suggest that BC morphological diversity may be underpinned by distinct genetic alterations.<sup>66–68 71 72</sup> In addition, intratumoural variation is not static and reversible changes in cancer cell properties can occur independent of hierarchical organisation.<sup>73–76</sup> Some cancer cells show the reversible transition between primary and metastatic sites, and expression of key cancer molecules is dynamically regulated during the process of carcinogenesis and tumour progression.<sup>77</sup>

In previous studies,<sup>1 69 78–81</sup> we have identified morphological spatial and temporal heterogeneity of BC between primary and metastatic tumour deposits. Some metastatic lesions feature a substantial degree of differentiation compared with that of their respective primary tumours. For instance, grade 3 primary tumours show grade 1 features at the metastatic sites, whereas others show apparent architectural differentiation in the form of DCIS like-areas; the so-called 'revertant DCIS'<sup>14</sup> as observed by our group and others<sup>14 82</sup> in up to 21% of cases. Tumour reversion, intratumoural heterogeneity, cancer cell plasticity and evolutionary adaptation have been proposed as mechanisms that promote drug resistance which pose a challenge to personalised cancer medicine. Knowledge of intratumour heterogeneity and mechanisms of tumour differentiation will provide new insights into neoplastic progression and lead to a better understanding of potential mechanisms of resistance to therapy. The molecular alterations underlying phenotypic changes in different types of BC<sup>69 83</sup> provide evidence for the dynamic nature of differentiation in BC and illustrate the importance of interpreting morphology in the correct context. Exact matching of the morphology and immunoprofile of the primary and metastatic tumours is not required to confirm the breast origin of the metastatic tumour provided that other possibilities are excluded.

## CONCLUSION

This review highlights the spectrum of BC differentiation and its impact on morphology which is reflected in the development of various tumour types with variable histological grade. BC differentiation underpins intertumour and intratumour heterogeneity and provides essential diagnostic and prognostic information. Deciphering the molecular mechanisms that control, facilitate or modify differentiation pathways is warranted to further advance our understanding of the biology of BC.

## Take home messages

- ▶ BC arises from a common epithelial progenitor or stem cell located at the terminal duct lobular units (TDLUs), which show a high degree of phenotypic plasticity.
- ▶ BC shows a broad spectrum of morphology, which stem from the complexity of BC carcinogenesis.
- ▶ Differentiation in BC determines the morphology, which can be measured using histological grade and tumour type.
- ▶ Histological grade, which measures the similarity to the TDLUs, reflects the degree of differentiation whereas tumour type reflects the type of differentiation.
- ▶ Understanding BC phenotypic differentiation facilitates the accurate diagnosis and histological classification.

Handling editor Cheok Soon Lee.

**Contributors** All authors contributed to the idea and writing the review.

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Provenance and peer review** Not commissioned; internally peer reviewed.

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