Triple negative breast cancers: Clinical and prognostic implications

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Abstract

Triple negative breast cancers are defined by the absence of oestrogen, progesterone and HER2 expression. Most triple negative cancers display distinct clinical and pathological characteristics with a high proportion of these tumours occurring at a younger age of onset and in African–American women. Triple negative tumours typically demonstrate high histological grade and are the most common breast cancer subtype in *BRCA1* carriers. In addition, many of the features of triple negative cancers are similar to those identified in the basal-like molecular subtype which has recently been characterised by gene expression profiling. Although the two groups overlap, they are not synonymous. Triple negative breast cancers are of pivotal clinical importance given the lack of therapeutic options. The prognostic significance of triple negative tumours remains unclear since the group is heterogeneous and worst prognosis seems to be mostly confined to those that express basal cytokeratins or epidermal growth factor receptor (EGFR). This review focuses on outlining the pathological, molecular, and clinical features of triple negative breast cancers, discusses its prognostic value and summarises current therapeutic approaches and future directions of research.

Introduction

The classification of invasive breast cancer for prognostic and predictive evaluation currently involves the assessment of histological criteria encompassing both morphology-based and immunohistochemical analysis. Traditional pathological parameters such as tumour size, axillary lymph node involvement and histological grade have been shown to correlate with prognosis [1,2]. Immunohistochemical markers such as the expression of hormone receptors (oestrogen (ER) and progesterone receptors (PR)) and the overexpression (or amplification) of HER2 provide therapeutic predictive value and are of key importance in guiding treatment selection [3]. Hormone receptor positive breast cancers account for around 75–80% of all cases. In contrast, HER2 positive tumours are identified in approximately 15–20%, with around half of these coexpressing hormone receptors [4]. The remaining 10–15% of breast cancers are defined by hormone receptor and HER2 negativity (i.e. triple negative cancers) [5–7]. Whilst effective targeted therapeutic modalities exist for women with hormone receptor positive and HER2 positive disease, chemotherapy is the only systemic therapy available for women with triple negative disease. The relatively aggressive clinical course, poor prognosis and lack of therapeutic options for this type of tumour have intensified current interest in this patient group.

Whilst the classification of human breast tumours on the basis of histological criteria has proven useful, there are a number of important limitations. Firstly, considerable variation in response to therapy and clinical outcome exists, even for tumours with apparent similarities in clinical and pathological characteristics. Secondly, this classification provides limited insight into the biology of breast cancer and the molecular pathways driving the disease. In recent years, gene expression profiling using microarray-based technology has provided a new way to classify human breast tumours and has resulted in the identification of clinically relevant molecular subtypes of breast cancer: luminal A, luminal B, HER2 overexpressing, normal breast tissue-like and basal-like cancers [8–11]. The difference in the gene expression patterns among the subtypes reflects basic alterations in the cell biology of the tumours. In addition, the molecular differences between the tumour subtypes have been associated with significant variation in clinical outcome [11, 12]. Basal-like breast cancers have been identified using gene expression profiling; however, no validated

consensus for the routine clinical identification of this subtype or other intrinsic subtypes of breast cancer exists. The features of basal-like breast cancers, as defined by microarrays, include the low expression of hormone receptor and HER2 amplicon genes. Therefore, in clinical specimens, basal-like breast cancers are often ER negative, PR negative and HER2 negative (i.e. triple negative cancers). The observed overlap between the triple negative phenotype and the basal-like breast cancer profile has provided new insights into this important subgroup of cancers, but has also created confusion.

Definition of triple negative versus basal-like breast cancers

The term "triple negative breast cancer" is now commonly used to define breast cancers that are ER, PR and HER2 negative using the clinical assays for these biomarkers [12]. In contrast, the term "basal-like breast cancer" refers to a molecular phenotype defined by an intrinsic gene set identified using mRNA gene expression profiling [8]. It is important to note that although these terms are often used interchangeably they are not the same entity. The original observation that the basal-like phenotype was associated with low expression of the ER, PR and HER2 genes in microarray analysis led to the conclusion that ER, PR and HER2 negativity in clinical specimens may be a surrogate for identifying this subgroup of tumours [13]. However, it has now become clear that not all basal-like tumours demonstrate a triple negative phenotype and conversely, not all triple negative breast cancers have a basal-like gene expression profile [6,14,15].

Immunohistochemical analysis of ER, PR and HER2 in basal-like breast cancers, classified according to the intrinsic gene list, has identified that at least 15% of these tumours are positive for one of these markers [6,11,16]. Conversely, not all triple negative cancers appear to be part of the basal-like cluster as defined by microarray analysis [6,13,15]. Furthermore, analysis of microarray based expression profiling suggests that in addition to the basal-like cluster, the triple negative group also encompasses tumours within the normal-like breast cancer molecular subtype [8–11,17,18]. Prevailing evidence has shown that the triple negative cancers are a heterogeneous group of tumours and those exhibiting a basal-like phenotype comprise only a subset of these cancers. Similarly, basal-like cancers as defined by microarray profiling, also show significant heterogeneity, with at least four distinct subclasses, named according to the expression of different gene modules identified using a novel algorithm and clustering approach [19–21]. In this review, the features of triple negative breast cancers will be discussed in parallel with those of basal-like cancers; however, the similarities and differences between these two groups will be highlighted where relevant.

To understand the definition of "basal-like" in the context of breast cancers, an appreciation of the normal cellular architecture of the breast is required. The human breast is composed of a branching ductal structure that ends in the functional unit of the breast, the terminal duct lobular unit. Breast ducts and acini comprise two cell layers: an inner luminal population of cells and a distinct outer layer of cells, juxtaposed to the basement membrane, termed the myoepithelium [22]. The luminal and myoepithelial cells can be distinguished by their immunophenotypic profile [23]. Luminal epithelial cells express low molecular weight cytokeratins (CK7, CK8, CK18 and CK19) in addition to other markers such as ER, PR and GATA3. It is this population of cells, within the lobular units, that proliferate and differentiate into secretory cells during lactation [24]. The myoepithelial cells are so-called because they share features of both epithelial and smooth muscle cells, and usually express high molecular weight cytokeratins (CK5, CK14 and CK17) in addition to markers specific to smooth muscle including the myofilament proteins smooth muscle actin and smooth muscle myosin heavy chain. The myoepithelial cells are often called basal cells because of their position adjacent to the basement membrane. However, the term basal has also become synonymous with cells that express high molecular weight or basal cytokerains [25]. In most tissues, the same population of cells is defined by those that occupy a basal position and those which express basal cytokeratins. However, in the human breast, basal cytokeratins are also expressed by a proportion of cells with a luminal or subluminal/supra-basal location [23]. The term basal may therefore be used in two separate contexts; firstly, to define myoepithelial cells, or secondly, to describe cells that express basal cytokeratins which may be found in either a luminal or basal location.

The original molecular subclassification of basal-like tumours inferred that this subtype reflected a myoepithelial cell origin. Counterintuitively, the vast majority of basal-like breast cancers appear to express luminal-type cytokeratins (such as CK8/18 and CK19) in conjunction with expression of basal cytokeratins [26]. Furthermore, only a small proportion

of basal-like tumours express classic myoepithelial markers such as smooth muscle actin and p63 [27]. These combined observations therefore suggest that basal-like tumours are not derived from differentiated myoepithelial cells. It remains unclear as to whether basal-like tumours are composed of cells that co-express both basal and luminal cytokeratins or are composed of two distinct lineages of epithelial cells. Recent advances in breast stem cell biology have identified phenotypic similarities between uncommitted cells with self-renewal potential and basal-like cancer cells, suggesting that basal tumours may be derived from mammary stem cells, although this is currently the focus of ongoing research [28–32].

Pathological features of triple negative breast cancer

Histopathological features

Triple negative cancers are predominately of high histological grade [33,34]. Several key additional histological features have been described in relation to basal-like cancers, when these tumours are defined on the basis of mRNA expression profiling or the expression of basal immunohistochemical markers. These features include increased mitotic activity and atypical mitotic figures, high Ki67 index, marked cellular pleomorphism, high nuclear atypia, high nuclear-cytoplasmic ratio, scant stromal content, central necrosis or central acellular zones, multiple apoptotic cells, pushing margins of invasion and stromal lymphocytic infiltration [26,27,35,36]. Many of these features are identified in high grade invasive carcinomas of no special type (NST), and although the morphological appearances may suggest a carcinoma is of basal type there are no specific histological features that can reliably distinguish basal-like cancers in routine clinical practice.

Whilst the majority of triple negative and basal-like cancers are high grade NST invasive carcinomas, several special histological types are also identified within this group including atypical and typical medullary carcinomas and metaplastic carcinomas [37–40]. Similarly, although most basal-like cancers are high grade, there are certain low grade breast carcinomas that also show a basal-like immunophenotype [33]. They account for around 10% of all basal-like cancers and are typically triple negative in addition to expressing basal type cytokeratins. These low grade histological subtypes include adenoid cystic carcinomas, low grade adenosquamous carcinomas and low grade spindle cell carcinomas [41,42]. In contrast to high grade basal-like cancers they demonstrate low mitotic activity, a reduced Ki67 index, minimal nuclear atypia and a better prognosis.

Immunohistochemical profile

Triple negative breast cancers are defined by ER, PR and HER2 negativity. In clinical practice, the assessment of oestrogen and progesterone receptor status is performed using immunohistochemistry, a process that requires strict quality control to ensure the accurate identification of endocrine responsive and non-responsive invasive breast carcinomas [43]. In contrast, HER2 status is typically assessed using a combination of immunohistochemistry and fluorescence *in-situ* hybridisation (FISH) [44]. Although approximately 15% of invasive breast cancers lack the expression of ER, PR and HER2, this depends on the clinical assays adopted and the criteria used to define ER, PR and HER2 negativity in each case.

Unlike triple negative cancers, the gold standard for identification of basal-like tumours remains gene expression profiling. Given that routine expression profiling is currently not possible in clinical practice, protein expression profiling using immunohistochemistry has been used to provide a similar molecular taxonomy of the disease [45,46]. Several attempts at defining immunohistochemical surrogate profiles for the basal-like subgroup have been made and although a wide variety of definitions have been proposed, achieving an optimal definition is a complex task and no currently accepted criteria have been established.

As previously discussed, the triple negative phenotype alone is not an adequate substitute for identifying basal-like breast tumours, as this group is likely to contain a mixture of basal-like and non-basal-like tumours and will miss the small subset of basal tumours positive for hormone receptors and/or HER2 [6,11,16]. Basal-like breast cancers commonly show heterogeneous and focal expression of basal high molecular weight cytokeratins such as CK5/6, CK14 and CK17 [6,8]. For this reason, the majority of immunohistochemical studies have used them to define basal-like tumours. However, the optimal cut-off to define basal cytokeratin positivity is not known and breast carcinomas have been deemed basal when they express basal cytokeratins in a single malignant cell or in 1%, 5% or 10% of malignant cells. In addition, it is unclear which basal cytokeratins should be used. The use of a single basal cytokeratin is likely to lead to a proportion of basal-like tumours failing to be identified. The original expression profiling

papers identified CK5/6 and CK17 in the basal-like subtype, but not CK14, which is commonly used in immunohistochemical studies [6,8]. Furthermore, although the majority of basal-like breast cancers express basal cytokeratins, a subset have been shown to be basal cytokeratin negative by immunohistochemistry [9,47].

In tissue-microarray studies, basal-like breast cancers have also shown high rates of expression of the epidermal growth factor receptor (EGFR) and c-kit [6,26,48]. When basal-like breast cancers have been defined using gene-expression profiles, EGFR and c-kit have been shown to be overexpressed in 57% and 31% of these tumours, compared to rates of 11% and 8% seen in non-basal-like tumours defined by a lack of CK5/6 staining [6]. Incorporation of EGFR and c-kit immunohistochemical markers into criteria for definition of the basal-like subgroup have therefore been considered.

One of the first definitions of basal-like breast cancers based on immunohistochemistry was proposed by Nielsen and colleagues where basal-like tumours were defined as those lacking both ER and HER2 expression and expressing CK5/6 and EGFR [6]. This panel was shown to have a specificity of 100% and a sensitivity of 76% for the identification of basal-like cancers and these criteria have subsequently been adopted by many other groups [26]. Others have restricted the definition to include PR (i.e. triple negative and CK5/6 and/or EGFR positive tumours) whilst some groups have used basal cytokeratins alone without consideration of triple negative status [48–53]. The heterogeneity of this subclass of tumours is such that no current immunohistochemical definition has been able to accurately identify all the cancers within this group.

Molecular features of triple-negative breast cancer

RNA expression profiling

The use of RNA expression profiling to characterise triple negative tumours has indicated that the majority of this group belong to the basal-like breast cancer subtype according to their intrinsic gene expression patterns [6,13,15]. In this context, the term "basal-like" has been used to describe this subtype as many of the genes expressed in these tumours are genes commonly identified in the basal or myoepithelial cells of the normal breast. These genes include important structural elements of basal epithelial cells (*CK5* and *CK17, P-cadherin, vimentin, metallothionein 1X*, *activating transcription factor 3* and *fatty acid binding protein 7*) and genes important in the interaction of basal cells with the extracellular matrix (*fascin, laminin, α6β4 integrin, matrix metalloproteinase 14* and *collagen type XVII alpha-1*) [8–11,18,32]. Other genes commonly overexpressed in basal-like tumours are those associated with many of the processes involved in tumourigenesis [54]. These include genes involved in signalling pathways (*MEK, ERK* and *PI3 kinases, AKT kinases* and *NF-κB*) and genes related to signal transduction (*c-kit, EGFR, caveolin 1* and *2, hepatocyte growth factor, c-fos* and *c-jun*) [18,32,55,56]. In addition, genes involved in cell proliferation (*MCM2, cyclin E1, STK6, topoisomerase IIα, MAD2L1, BUB1, CDC2*), cell migration (*TGFβ2*) and heat shock proteins (*αB-crystallin* and *Hsp27*) have all been shown to be up-regulated in this tumour subtype [8,9,11,18,32,57]. In contrast, genes which encode for characteristic proteins of luminal epithelial cells such as hormone receptors (*ER* and *PR*) and GATA transcription factors (*GATA3*) are frequently downregulated in these tumours, as are genes located in the HER2 amplicon at 17q21 [8,9,11,18,32].

Analysis of the genes differentially expressed in ER negative tumours using a novel algorithm has identified four distinct clusters, named according to the expression of characteristic gene modules: cell cycle and cell proliferation genes (CC+), immune response genes (IR+), cell cycle and immune response genes (CC+/IR+) and extracellular matrix genes (ECM+) [19]. Basal-like tumours, as defined by the intrinsic gene set, are distributed across each of these four clusters, highlighting the underlying hetereogeneity of this molecular subtype [19]. In particular, medullary breast cancers, which typically show basal-like features, cluster within the CC+/IR+ group [19].

Since the pivotal gene expression profiling publications, subsequent studies have identified some differences in the details of the intrinsic gene set generated by hierarchical clustering [42]. Of note, the number of basal-like cancers included in the original studies were small; the Perou paper was based on 38 invasive cancers of which only six had basal-like features, and the subsequent study by Sorlie examined 76 invasive cancers which included seven basal-like tumours [8,9]. Despite this, a basal-like subtype has been reliably identified in multiple series, including a meta-analyses where various microarray experiments done using different platforms were combined [8–11,18]. Importantly, studies consistently show that the majority of triple negative cancers are found within a select gene cluster and that most do express genes

that overlap with the basal-like intrinsic gene set [6,8–11,13].

Genomic profiling

Triple negative and basal-like tumours have both been characterised using comparative genomic hybridisation (CGH) which has shown that the majority demonstrate increased DNA-copy number changes compared with other breast cancer subtypes suggesting a greater degree of genetic instability [58–64]. The application of array-CGH to breast cancers characterised as triple negative using immunohistochemistry and basal-like through expression profiling has revealed that these tumours frequently show low-level gains and deletions [58,59,62]. Characteristic cytogenetic changes that have been reported include amplifications at 7p11.2 involving the *EGFR* gene, 7q31 affecting caveolin 1 and 6p21-p25 which harbours several candidate oncogenes including *DEK, E2F3, Notch4, Pim1 and CCN*D3 [56,58,63,65]. Although a high degree of genetic complexity has been reported, it remains uncertain as to whether these changes reflect the high histological grade of the majority of these tumours. In a recent study, basal-like tumours, which were defined immunohistochemically by CK14 positivity, demonstrated a lower frequency of genomic alterations compared to grade-matched controls, although distinct alterations were observed such as losses at 16p, 17q, 19q and Xp [52]. Furthermore, an array-CGH analysis of 171 primary breast cancers has identified a subclass of breast tumours with low genomic instability characterised primarily by oestrogen receptor negativity and a relative over representation of a basal-like gene expression profile [66].

Genome wide single nucleotide polymorphism (SNP) arrays have also revealed characteristic changes in the basal-like subtype of tumours. Basal-like cancers classified on the basis of expression array data demonstrate a rate of loss of heterozygosity (LOH) which is around two to three times higher than that of other subtypes of breast cancer [67]. In particular, loss at 5q, where many checkpoint, DNA-repair and tumour suppressor genes are located including *MSH3, RAD17, APC, RAD50* and *XRCC4*, was identified in 100% of basal-like tumours but never in luminal or other subtypes [67].

Finally, a significant proportion of triple negative and basal-like carcinomas are also associated with sporadic *p53* mutations and/or p53 protein overexpression [9,34,46,50,68]. In the original gene expression profiling study by Sorlie and colleagues, 82% of basal-like breast cancers had *p53* mutations compared with only 13% in the luminal A subtype [9]. Given the high rate of genomic instability identified in the basal-like subtype, a lack of functioning p53 is probably critical in promoting cell survival in this context.

Despite the identification of characteristic genomic changes in the triple negative and basal-like subtypes of breast cancer, it is important to highlight that significant heterogeneity remains even within these tumour subgroups. Mutational analysis at the whole genome level in breast cancer has only been done in ER negative cases and considerable heterogeneity among the mutated candidate cancer genes identified in this group has been shown [69,70].

MicroRNA profiling

MicroRNAs (miRNAs) are small non-protein coding single stranded RNAs of ~22 nucleotides that function as gene regulators [71]. They govern diverse biological processes and may influence almost every genetic pathway through their control of hundreds of mRNA gene targets. Altered expression of miRNAs is associated with various human cancers and miRNAs appear to function as tumour suppressors and oncogenes in different contexts [72]. The first described oncogenic miRNA was the miR-17-92 cluster which acts as a key regulator of the G1/S phase cell cycle transition [73,74]. Over-expression of the miR-17-92 cluster, which has been identified in many human cancers including breast cancer, has been shown to deregulate normal cell cycle progression, and drive a proliferation phenotype [73].

MicroRNA microarrays have been useful in determining tissue specific signatures of miRNA genes in normal tissues and cancers. Expression profiles of relatively few miRNAs (~200 genes) have been shown to accurately classify human cancers, with tumours originating from various tissues clustering solely on the basis of their miRNA expression profiles [75]. MicroRNA expression profiling has also been able to classify breast cancers into relevant prognostic molecular subtypes, as has been shown previously with mRNA gene expression profiling [76]. In this context, certain miRNAs have been shown to classify basal versus luminal tumour subtypes. A quantitative RT-PCR analysis of miRNA expression from breast cancer biopsies has also revealed that miRNA expression can be used to classify ER status [77]. Increasing evidence now indicates that the differential expression of miRNAs may also correlate with distinct clinicopathological features and can be used to guide a patient's overall prognosis [78,79]. In the future, miRNA expression profiling may prove to be a

powerful tool for clinicians for the identification and characterisation of triple negative tumours.

Common features with BRCA1 related cancers

There is now strong evidence to suggest a link between *BRCA1* pathway dysfunction and basal-like breast cancers. Basal-like cancers and tumours that arise in *BRCA1* germline mutation carriers share many morphological and immunohistochemical characteristics. The vast majority of cancers in *BRCA1* germline mutation carriers, especially those in women under 45 years of age, are of high histological grade, have a triple negative phenotype and express basal cytokeratins and EGFR by immunohistochemistry [50,80–82]. The observed similarities have led investigators to suggest that the use of cytokeratin staining in combination with ER and morphology provides a predictor of *BRCA1* mutation status that may be useful in selecting patients for *BRCA1* mutation testing [80].

Parallels between the molecular features of basal-like tumours and cancers arising in *BRCA1* germline mutation carriers are also evident. The majority of breast cancers in those with *BRCA1* germline mutations demonstrate a basal-like expression profile and cluster within the cell cycle and immune response subclass (CC+/IR+) of ER negative breast cancers, suggesting that similar biological pathways are important in these two tumour subtypes [10,19,83,84]. Common cytogenetic abnormalities have been identified in the two groups, including loss of 5q [58,60, 61,83,85,86]. In addition, *p53* is commonly mutated in both subtypes, although the spectrum of mutations in BRCA1 related tumours is distinct from those observed in sporadic basal-like tumours [87]. Furthermore, frequent loss of X-chromosome inactivation has been observed in both basal-like and *BRCA1* breast tumours [88,89]. All of these similarities support the notion that even in the absence of a germline *BRCA1* mutation, *BRCA1* pathway function is compromised in basal-like breast cancers.

Current research is now focussing on the link between sporadic basal-like breast cancers (those occurring in women without germline *BRCA1* mutations) and dysfunction of the *BRCA1* pathway [83, 90,91]. Somatic *BRCA1* mutations are very rare in breast cancer and the mechanisms leading to *BRCA1* dysfunction in sporadic basal-like breast cancers are not well understood [92]. *BRCA1* mRNA and protein expression levels have been shown to be significantly lower in triple negative tumours that demonstrate a basal-like phenotype [46,91]. Methylation of the promoter of *BRCA1* has been identified particularly in medullary and metaplastic subtypes of basal breast cancers [91,93,94]. However, a recent study has shown that when comparing sporadic invasive NST carcinomas with and without basal-like features, a low prevalence of *BRCA1* gene promoter methylation was identified in both groups [95]. An alternative mechanism to explain the low levels of BRCA1 expression in sporadic basal-like carcinomas may involve ID4, a negative regulator of *BRCA1*, which has been found to be overexpressed in basal-like tumours [91].

The role of *BRCA1* inactivation as a cause or consequence of the development of basal-like cancers is currently unknown. *BRCA1* is known to be important for normal mammary gland development and is also involved in regulating the ER pathway [96,97]. *BRCA1* can directly modulate expression of ER and is required for the conversion of ER negative to ER positive cells [98]. This concept supports the notion that *BRCA1* may regulate mammary progenitor cell fate [98,99]. Loss of *BRCA1* may affect normal differentiation in the mammary gland and the development of tumours displaying a triple negative or basal-like phenotype. Ongoing research in this field is needed to fully elucidate the mechanisms involved.

Clinical features of triple negative breast cancer

Genetic and epidemiological risk factors

In addition to high-penetrance mutations such as those in *BRCA1*, other lower-penetrance genes may increase the risk of developing particular tumour subtypes either alone or in combination with environmental factors. Recent genome-wide association studies have identified novel breast cancer susceptibility loci involving SNPs in CASP8, FGFR2, TNRC9, MAP3K1 and LSP1 [100,101]. The majority of these susceptibility loci appear to have stronger associations with ER positive rather than ER negative disease, suggesting that common genetic variants can influence the pathological subtype of breast cancer [102]. The current studies, however, are under-powered to detect susceptibility loci in less common tumour subtypes such as triple negative and basal-like tumours, although ongoing work may identify additional susceptibility loci for these tumour subtypes in the future.

Epidemiological studies examining risk factors for breast cancer have not been designed to explore risk factors in select molecular subgroup populations. However, repeat analysis of risk factors stratified by tumour subtype have identified some interesting

findings, suggesting that several traditional risk factors have different effects in basal-like versus luminal breast cancers defined by immunophenotyping. High parity combined with lack of breast feeding, younger age at first birth, early onset menarche and abdominal adiposity appear to relate to an increased risk of basal-like breast cancers [103,104]. In contrast, risk factors for luminal disease are typically those reported in previous epidemiological studies such as nulliparity and later age at first birth [103]. Future epidemiologic studies will need to incorporate analyses of molecular subtypes in order to fully appreciate the impact of traditional risk factors in different contexts.

Patient age and ethnicity

Several studies have suggested that the prevalence of the triple negative and basal-like subtypes varies according to age and race. A large cohort study of 1601 breast cancer patients (including 180 triple negative cases) showed that the mean age at diagnosis was younger for those women with triple negative tumours (53 versus 58 years, $P < 0.0001$) [33]. In gene expression and immunohistochemistry studies, the average age of patients with basal-like cancers appears to range from 46 to 54 years [5,49,104,105]. Two of these studies identified that patients with basal-like breast cancers have the lowest average age across all of the intrinsic molecular subtypes, whereas the Polish Breast Cancer Study found no significant difference in patient age between the different breast cancer groups [49,104,105].

Triple negative and basal-like breast cancers occur more commonly in African–American women, particularly those who are pre-menopausal [7,49, 103,106,107]. In a single institutional study of 2230 women, the triple negative phenotype was more common in African–Americans compared with non-African–American women (20.8% versus 10.4%, $P < 0.0001$) [107]. In the Carolina Breast Cancer Study where basal-like cancers were defined using immunohistochemistry, 39% of breast carcinomas in premenopausal African–Americans were basal-like, as compared to 14% in postmenopausal African–Americans and 16% in non-African–Americans of any age ($P < 0.001$) [49]. It remains uncertain as to whether these racial differences are related to genetic factors, environmental factors or a combination of both.

Stage, patterns of relapse and metastases

Previous studies of basal-like breast cancers (defined by triple negative status plus EGFR or CK5 positivity) have shown that they do not differ significantly from other types of breast cancer in terms of stage at diagnosis [49]. However, it has been shown that the basal-like subgroup more frequently present as interval breast cancers compared to those cancers detected through screening. A small study of 95 interval cancers diagnosed in Norway reported that cancers detected outside of screening were more likely to be hormone receptor negative and express cytokeratin 5/6 [108].

The locoregional relapse rate for triple negative cancers appears to be identical to that of other molecular subgroups after conservative surgical management; however, the triple negative phenotype appears to be associated with a higher rate of distant metastases [109]. Furthermore, tumours defined by basal cytokeratin expression do appear to show specific patterns of distant relapse with a higher propensity for visceral metastases to the brain and lungs and a lower incidence of metastases to the liver, bone and axillary lymph nodes [49,51,110]. In one study of basal-like cancers defined by CK5/6 expression, the correlation between tumour size and number of positive lymph nodes was weak compared with tumours that did not express CK 5/6 [50]. Taken together, these findings do not suggest that a more radical approach to local or axillary surgery is warranted, although distinct mechanisms of metastatic spread may be important in this tumour group.

Triple negative breast cancers also show a difference in the timing of relapse. The risk of recurrence appears to be highest in the first 5 years after diagnosis, with relatively few systemic recurrences occurring after this period. A single institutional cohort study of triple negative cancers showed that distant recurrences peaked at approximately 3 years after diagnosis in the triple negative group [33]. In contrast, the recurrence risk for the non-triple negative group remained constant over time. This pattern of recurrence has been previously identified in women with ER positive disease, where there is a continued risk of late relapse [111]. In particular, for women with triple negative disease, a substantial number appear to be cured if they remain recurrence free for the first several years after diagnosis.

Prognosis

The majority of triple negative breast cancers are characterised by an aggressive clinical history, shorter survival and a relatively high mortality rate [7, 33,109,112]. The molecular signature of basal-like breast cancers has also been associated with a poor

clinical outcome [9,11]. A recent comparison across different gene expression prognostic signatures has revealed that almost all patients with basal-like breast cancers are classified as having high recurrence scores, poor 70 gene profiles, and activated wound response signatures [17]. Regardless of the different gene sets used to classify these patients, they all appear to show significant agreement in the outcome prediction for this subtype. Although most basal-like breast cancers defined by expression profiling demonstrate a poor prognosis, a subgroup of ER negative tumours with a good prognosis has been identified on the basis of an immune response gene expression cluster (IR+) [19,113–115].

The majority of basal-like breast cancers as defined by immunohistochemical profiles also appear to confer a worse prognosis [27,46,49–51,116,117]. The expression of basal markers (basal cytokeratins and EGFR) may separate a clinically significant subgroup within the triple negative cancers, and in some studies, expression of basal cytokeratins and/or EGFR has identified a subgroup of cancers that display a poor prognosis regardless of the expression of ER or PR [6, 34,112,117–119]. A recent analysis of 4000 cases compared the prognostic significance of the triple negative phenotype with and without the addition of basal markers (CK 5/6 and EGFR) [119]. The poor prognosis of the triple negative phenotype was conferred almost entirely by those tumours positive for basal markers, and the addition of basal markers had superior prognostic value compared to relying on the triple negative phenotype alone [119]. Some studies have shown that basal cytokeratins are independent markers of poor prognosis after adjustment for variables such as age, tumour grade, tumour size and lymph node status [27,53,116–119]. Other studies have shown that the prognostic significance of the basal subtype disappears when evaluated alongside other high risk variables such as ER negativity, HER2 positivity and grade 3 status [5,47,120]. In addition, a number of low grade histological subtypes of basal-like tumours have been described and these are consistently associated with improved prognosis [41,42]. Difficulties in the interpretation of studies arise because of differences in the criteria used to define the basal-like subtype. In addition, many studies have grouped patients together who were undergoing different treatment protocols. Future large prospective clinical trials will be needed to provide conclusive evidence for the prognostic impact of the basal-like immunophenotype independent of traditional clinicopathological variables and the type of adjuvant therapy received.

Therapeutic implications for triple negative breast cancer

Response to therapy

Chemotherapy remains the only systemic treatment option available for patients with triple negative breast cancers and a number of small studies have documented that these tumours do appear to be chemosensitive. In a neo-adjuvant study involving 255 patients with triple negative disease receiving either anthracycline or anthracyline and taxane-based regimens, the pathological complete response rate of triple negative tumours was significantly higher than that identified for non-triple negative cancers (22% versus 11%, $P = 0.034$) [121]. Other studies have shown similar findings for basal-like cancers defined through gene expression profiling or immunophenotyping [16,122]. Importantly, however, the study by Rouzier and colleagues, which defined basal-like tumours using expression profiling, showed that molecular class was not an independent predictor of chemotherapy response after accounting for the high grade, high proliferation rates and hormone receptor negative status of these tumours [16].

Tumours with defective DNA repair pathways such as those with *BRCA1* deficiency, may be highly sensitive to DNA damaging agents such as platinum based drugs. [83,91,123–125] Given that many triple negative tumours display similarities to the BRCA1 phenotype, further insights into chemotherapy responsiveness in this tumour group may be gained from these studies. In a recent small neoadjuvant trial of women with *BRCA1* mutations and triple negative breast cancer, nine out of ten women had complete pathological responses to single agent cisplatin. [126] Conversely, other evidence suggests that tumours with *BRCA1* mutations may be less sensitive to taxanes [127,128]. In keeping with these findings, triple negative cancers do not appear to show increased sensitivity to taxanes compared with non-triple negative cancers, despite the fact that they demonstrate a high prevalence of *p53* gene mutations which have been shown in some studies to predict taxane responsiveness [129,130]. Clinical trials testing the efficacy of platinum agents and taxanes in the management of patients with triple negative cancers are currently underway [131]. These include the "Triple Negative Trial" which is a phase III randomised clinical trial comparing carboplatin with docetaxel in women with triple negative disease [132].

Pathway based approaches and targeted therapies

Triple negative tumours and basal-like breast cancers defined through immunophenotyping and gene expres-

sion profiling have been found to overexpress EGFR in up to 60% of cases, although the gene is rarely mutated in breast cancers [6,26,34,48,60,133]. In particular, the metaplastic subtype of triple negative tumours has shown EGFR gene amplification in up to 25% of cases [65,134]. Basal-like breast cancer cell lines also appear to depend on EGFR for growth and proliferation [135]. EGFR signalling has been successfully targeted in other cancer types using humanised anti-EGFR monoclonal antibodies (e.g. cetuximab) and EGFR tyrosine kinase inhibitors (e.g. gefinitib and erlotinib) [136,137]. EGFR gene amplification has been shown to correlate with response to anti-EGFR targeted therapies, suggesting a possible benefit for patients with triple negative breast tumours [138,139]. The activity of cetuximab, in combination with platinum agents or taxanes, is currently being investigated for the treatment of triple negative breast cancers in the metastatic setting [131].

The overexpression of c-kit has been identified in around 30% of basal-like breast caners defined through immunohistochemistry [5,6]. This has raised the possibility of using imatinib, a c-kit and platelet derived growth factor receptor tyrosine kinase inhibitor, as a targeted therapy in these tumours. Mutations in the *KIT* gene have previously been shown to be strong predictors of response to imatinib in gastrointestinal stromal tumours (GIST). However, in contrast to GIST, mutations in the KIT gene are infrequent in breast cancer [140]. Activity of imatinib has been demonstrated in breast cancer epithelial cell lines; however, no activity was seen in a recent phase II trial of 16 patients with metastatic breast cancer, although only one of these patients was positive for c-kit using immunohistochemistry [141,142].

Dasatinib is a multitargeted kinase inhibitor that inhibits src and abl. It has recently been shown to be effective in breast cancer cell lines with a basal-like phenotype [143]. Using gene expression profiling data from a panel of breast cancer cell lines, a six gene predictor of response to dasatinib has recently been developed [144]. Furthermore, this gene set has been observed in primary breast tumour samples preferentially lacking ER, PR and HER2 [144]. A small phase II nonrandomised study is now investigating the efficacy of dasatinib in women with advanced triple negative breast cancers [131].

Poly (ADP-ribose) polymerase-1 is an abundant nuclear enzyme essential to the repair of double strand breaks by the nonhomologous DNA repair pathway. Defects in the repair of DNA damage through homologous recombination, which occur in the setting of *BRCA* deficiency, appear to sensitise cells to PARP inhibition [123,125]. Encouraging preliminary results have been seen in initial phase I clinical trials of PARP inhibitors which have included patients with *BRCA1* deficient tumours. Several phase II studies of PARP inhibitors, either alone or in combination with DNA damaging agents, in both *BRCA1* carriers and women with triple negative breast cancers, are currently underway.

Other options for the use of targeted therapies in this subtype of breast cancer include the application of antiangiogenic strategies such as monoclonal antibodies against the vascular endothelial growth factor (VEGF) receptor (e.g. bevacizumab) or multikinase VEGF receptor inhibitors (e.g. sunitinib). In a phase III trial of over 700 women with metastatic breast cancer randomised to receive paclitaxel with or without bevacizumab, a significant improvement was seen in progression free survival with the addition of bevacizumab, including the subgroup of patients with triple negative disease [145]. A recently reported phase II study of sunitinib has also identified a response rate of 15% in the pretreated triple negative subset of patients [146]. Specific phase II studies investigating the roles of bevacizumab and sunitinib, in addition to chemotherapy, are also being investigated in the triple negative setting [131].

Finally, the *ras-raf* -mitogen activated protein kinase (MAPK)/extracellular signal regulated kinase (ERK) kinase (MEK) pathway and the phosphoinositide 3-kinase (PI3K)-PTEN-AKT signalling pathway, both of which play key roles in signal transduction networks promoting tumour initiation and progression, may be important therapeutic targets for triple negative and basal-like tumours. Recent *in-vitro* evidence suggests that basal-like breast cancer cells are particularly susceptible to growth inhibition by small molecule MEK inhibitors [147]. In addition, high expression of the PI3K pathway and lower levels of the tumour suppressor PTEN have been demonstrated in basal-like cancers, with PI3K inhibition shown to lead to basal-like cell growth arrest *in-vitro* [148].

Conclusions

Triple negative breast cancers are a heterogeneous subset of tumours grouped together on the basis of their lack of hormone receptor and HER2 expression. Although they demonstrate similarities in terms of pathological, molecular and clinical characteristics, they do not represent a uniform clinical entity. They share common features with the basal-like molecular subtype, but the two groups remain distinct. The basal-like breast cancers are themselves a heterogeneous

group, and triple negativity should not be used as a surrogate marker for their identification and characterisation. The clinical relevance of triple negative breast cancers is intensified by the relatively poor prognosis, although this is probably confined to a subgroup that expresses basal cytokeratins and/or EGFR, and paucity of treatment options for this patient group. Given that they account for only 15% of all breast cancers, substantial collaboration will be required to drive meaningful research in this area. Numerous therapeutic targets are emerging as potential options; however, the underlying heterogeneity of this tumour group will undoubtedly lead to different treatment recommendations in different contexts. The introduction of new technologies such as high-throughput genome sequencing, miRNA profiling and the integration of this information into projects such as the International Cancer Genome Consortium will undeniably further our understanding of this subtype and lead to an improvement in the classification of breast cancers into biologically and clinically relevant groups. The application of targeted therapies based on biomarker selection in appropriately designed clinical trials will be the key future goal, in order to identify patients with triple negative disease most likely to benefit from selected treatments.

Conflict of interest statement

None declared.

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