Demystifying basal-like breast carcinomas

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J Clin Pathol 2007;60:1328–1332. doi: 10.1136/jcp.2006.041731

“Basal” breast cancers are dominating the breast research literature at present and pathologists are under increasing pressure to evaluate for such a phenotype by their surgical and oncological colleagues. There is also much confusion about how to assess cancers, which immunohistochemical markers to use, what meaning and benefit this provides, and what the surgeons and oncologists will do with the information. Much remains to be done to answer all these questions but here we try to shed light on some of the issues and suggest what is still to come.

Since the publication of two seminal papers using gene expression profiling to sub-classify breast cancer,^{1 2} there has been an increasing reference in the literature to “basal” breast cancer. To date, there is no agreement on the definition of these tumours, nor is there a single term used to describe them; “basal”, “basal-like”, “basaloid” and “tumours with a basal/myoepithelial phenotype” all appearing in the literature. In this review we attempt to clarify some of the issues related to the pathology, biology, identification and management of these cancers.

THE NORMAL BREAST

The mammary gland is an endocrine responsive glandular tissue composed of 15–20 irregular segments and approximately 10–12 major collecting ducts opening up at the nipple. At the distal end are the terminal duct lobular units (TDLU), believed to be the functional units of the breast and the place where most pathology is thought to arise. This branching duct-lobular system is lined by two main types of cells; the inner luminal secretory cells and the outer incomplete layer of contractile myoepithelial/basal cells, which are arranged in a basket-like network. The epithelial cells are surrounded by and separated from the stroma by the basement membrane.³

The basal/myoepithelial cells are heterogeneous,⁴ and may appear spindle shaped or cuboidal depending on the anatomical location and hormonal status. The “myoepithelial cell” is so called because the cells express epithelial specific as well as muscle specific proteins such as smooth muscle actin (SMA), smooth muscle myosin, calponin and caldesmon. In addition, they also express p63, CD10, maspin, 14-3-3-sigma and s100 protein among others.

It was the classical work of Moll and colleagues⁵ that led to the identification of sub-types of keratins expressed in simple and stratified epithelia and cancers. With the development of cytokeratin specific antibodies,⁶ it became clear that high

molecular weight (HMW) cytokeratins such as 5/6 and 14 are found in the basal layer of stratified epithelia, hence these came to be known as “basal cytokeratins”.^{5 7} This is not a problem when the cytokeratins stain the cells that sit in a basal position next to the basement membrane, as in the salivary gland, but it does cause problems in the breast. While there is no doubt that the myoepithelial cells do express these HMW “basal” keratins, paradoxically, they are also expressed in a small proportion of cells within the inner, luminal cell compartment.⁸ Hence, although the term “myoepithelial” and “basal” is often used synonymously in the literature, it does not imply that the “basal” breast cancers have necessarily evolved from the myoepithelial compartment since a proportion of normal cells within the luminal compartment also have a “basal”, or more pedantically, a mixed basal and luminal phenotype.

Putting aside that anomaly and the possibility of confusion, what do we know about tumours that have come to be designated as “basal” or “basal-like”?

HISTORICAL PERSPECTIVE

There is a common misconception in the literature that the “basal” breast cancer was *discovered* by microarrays.^{1 2 9} In truth, the microarray experiments, to their credit, brought this tumour type out of the closet and into the frontline where it belongs. During the 1960s, papers by Wellings and Roberts,¹⁰ Sarkar and Kallenbach¹¹ and Murad and Scarpelli¹² demonstrated that a proportion of breast cancers had a myoepithelial phenotype on ultrastructural (electron microscopic) examination. It was the classical experiments of Moll and colleagues,^{5 7} looking at cytokeratin profiles in simple epithelia and cancers, together with a number of further reports^{13–23} investigating specific cytokeratin profiles of normal, benign and malignant breast tissue, which showed that 2–18% of all breast cancers and up to 25% of grade III tumours expressed HMW cytokeratins and hence had a “basal/myoepithelial phenotype”. It was also recognised that these tumours were negative for oestrogen receptor (ER), progesterone receptor (PgR) and HER2.^{16 22} The “basal” phenotype had therefore already been described in the literature prior to microarray experiments, although it had not made it into the mainstream classification system for breast disease.

THE MOLECULAR REDISCOVERY OF BASAL-LIKE TUMOURS

Microarray technology has been an incredible advance.^{1 2 24} It has made it feasible to detect and

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Accepted 26 April 2007
Published Online First
11 May 2007

to quantify simultaneously the expression of a large number of genes and obtain a broader picture of pathways and networks that are up or down regulated in the tumour cell. This is a major advance from the reductionist, one gene investigation that had predominated in the previous two decades since cells work in complex interacting networks rather than as solitary entities. The gene expression profiling studies^{1 2 9 24-26} in breast cancer have divided the tumours into five groups: luminal A (ER+); luminal B (ER+); HER2 over expressing; normal breast-like; and basal-like/basal/basaloid type. The “basal” group of tumours was characterised in these experiments by expression of genes related to myoepithelial cells, such as calponin 1, laminin, caveolin, keratin 5, and keratin 17. They were also found generally not to over express HER2 and were negative for ER and PgR. This much was already known. The finding that rocketed this subgroup into mainstream thinking was the observation in the study by Sorlie *et al*² that “basal” tumours had an extremely bad prognosis, even worse than the HER2 positive cancers. In fact, all patients in the “basal” group had died within 4 years of diagnosis.

Since then, numerous studies have attempted to further define the pathology, biology, clinical features and therapeutic options for this interesting subgroup of tumours.

PATHOLOGY

Tumours expressing basal/myoepithelial markers such as SMA, S100 protein or CK14/CK5-6 have been shown to have distinct morphological and cytological features that are different to non-basal cancers.^{18 19 27-29} They are more likely to contain central areas of scarring and often show areas of necrosis (sometimes referred to as an invasive comedo pattern). They have pushing or partially pushing margins with a peri-tumoural lymphocytic infiltrate and may have a syncytial growth pattern. The tumours may also contain areas of spindle cell and squamous metaplasia. Basal cancers are usually high grade with a very high mitotic index, and the cells show a high nuclear to cytoplasmic ratio (fig 1A–D).

The features described above clearly show that there is an overlap with medullary carcinoma, a rare, diagnostically difficult and controversial subtype of breast carcinoma.^{30 31} The medullary phenotype is also associated with BRCA1 germ-line mutation,^{32 33} and as might be expected, a high proportion of medullary and BRCA1 associated cancers show a “basal” phenotype.³⁴⁻³⁷ The fact that spindle cells and squamous metaplasia are also associated with this phenotype²⁷ suggests that metaplastic breast cancers may be part of the spectrum of “basal” tumours. Indeed this is the case.³⁸⁻⁴¹ Metaplastic carcinomas are an interesting group of tumours that show a heterogeneous appearance, ranging from purely epithelial differentiation (homologous type) to mixed epithelial and mesenchymal differentiation (heterologous type), and can be distinguished from the common type of ductal carcinomas on morphology as well as immunophenotyping including the “basal” markers.

The immunophenotype of “basal” cancers is interesting in that they are often, though not invariably, triple negative (ER, PgR and HER2 negative) but over-express HER1 (epidermal growth factor receptor, EGFR) in a high proportion (50–70%) of cases. Overall, the morphology and immunophenotype is sufficiently different to be useful in diagnostic practice³⁵ in alerting the pathologist to the likelihood of the cancer being of a “basal” subtype. Further staining for high molecular weight cytokeratins can be carried out to confirm the suspicion. It has to be added that there is no consensus as to which cytokeratins or other “basal” markers should be used. In our laboratory, we use CK14 and CK5/6 as the minimal panel (with ER, PgR and HER2 being carried out already as a standard breast panel).

Nielsen *et al*⁴² proposed a panel comprising ER, HER2, CK5/6 and EGFR based on expression profiling data. This approach is also perfectly valid as it uses a combination of negative (ER and HER2) and positive staining (CK5/6 and EGFR). Currently many laboratories do not use EGFR, and since the cytokeratins are relatively easy to use and establish in clinical practice in a wide range of laboratories, we have opted for this preference.

It has been shown in studies carried out on behalf of the Breast Cancer Linkage Consortium that a combination of morphology, negativity for ER and “basal” keratin positivity could provide a powerful predictor tool for BRCA1 mutation status and hence may be useful in selecting patients for BRCA1 mutation testing.³⁵ Many women who harbour mutations in BRCA1 gene do not have a family history of breast and/or ovarian cancer. Hence, finding a cancer in a young woman with morphological features and immunophenotype indicating a medullary-like/basal-like tumour can help alert doctors to the possibility of a familial predisposition. Clinical geneticists have been working hard to develop risk assessment algorithms to help identify women who may benefit from testing, and the incorporation of pathology data into such algorithms has been shown to be beneficial.^{43 44}

Recently, Bryan *et al* showed immunohistochemical evidence (hormonal receptors, HER2, EGFR and cytokeratin staining) of high grade ductal carcinoma in situ with an analogous immunophenotype to that of invasive basal-like carcinomas.⁴⁵ Likewise, Paredes *et al*⁴⁶ showed that P-cadherin and CK5 are able to identify an in situ form of basal-like ductal carcinoma. The in-situ counterparts of the invasive carcinoma subtypes (“basal-like” type, ERB-B2 type, and luminal-type) identified by microarray experiments have also been described by Hannemann *et al*⁴⁷ and Livasy *et al*.⁴⁸ The differences in the subtypes of invasive carcinoma noted on expression profiling are also reflected at the genomic level using comparative genomic hybridisation.⁴⁹ As far as we are aware, genomic analysis of the “in-situ basal” lesions has not been reported to date.

DO “BASAL” CANCERS ARISE FROM BASAL CELLS?

There is considerable morphological and molecular data to support the idea that the pathways for the development of high grade and low grade breast cancers are separate from the outset.⁵⁰⁻⁵⁵ In other words, that the de-differentiation of low grade carcinoma into a high grade carcinoma is a rare event. The data on “basal” cancers appears to follow this general principle in that the “basal” cancers fall into the high grade pathways and the phenotype can be identified at the in-situ stage. Not surprisingly, it has been postulated that “basal” cancers probably arise from the “basal/myoepithelial” cell compartment due to the similar immunohistochemical profile. This is clearly an oversimplification since we know that a small proportion of cells in the luminal compartment express “basal” markers such as HMW cytokeratins. Others have suggested that the ability of “basal” cancers to show a mixed basal/luminal phenotype implies an origin from stem cells. We believe that this is also an oversimplification, since little is currently known about the plasticity of different cell populations within the differentiation lineages from stem cell to adult cell populations. It is equally plausible that a luminal cell, having acquired mutations during the evolution to a cancer cell, becomes capable of expressing a wide range of molecules giving rise to divergent differentiation as seen also in metaplastic carcinomas.

So the simple answer to the question is: “Nobody knows!” Our knowledge in the field of stem cell and normal cell biology^{56 57} is expanding rapidly, and no doubt light will be shed on this area in the near future.

Figure 1 (A) Basal-like breast cancer with pushing margins and central necrosis (H&E, $\times 40$). (B) Tumour necrosis (H&E, $\times 400$). (C) Lymphocytic infiltrate (H&E, $\times 400$). (D) Abnormal mitosis (H&E, $\times 400$). (E) Diffuse CK 14 positivity ($\times 100$). (F) Focal CK 14 staining ($\times 100$).

CLINICAL ASPECTS

“Basal” breast cancers have been described across a number of different populations throughout the world and the gene expression signature has been identified across independent data sets and platforms.^{9 26 49 58} They occur at a slightly younger age than other grade III ductal carcinoma, with a mean of 49.9 years versus 53.9 years in the study of Fulford *et al.*⁵⁹ Despite being generally high grade and ER negative, they are less likely to have nodal metastases.^{29 59} This combination of a high grade, ER negative, node negative cancer has also been reported in the setting of familial BRCA1 associated tumours.^{33 35 60}

The original expression profiling studies of Sorlie *et al.*¹² suggested a dismal prognosis for “basal” cancers. Even putting aside the pure medullary carcinomas and metaplastic breast cancers, the ductal carcinomas with a “basal” phenotype appear to be morphologically heterogeneous. Jones *et al.*¹⁶ performed comparative genomic hybridisation analysis on 43 grade III invasive ductal breast carcinomas positive for basal CK14 along with 43 CK14-negative controls, all with clinical follow-up. Unsupervised hierarchical clustering showed four clusters within the CK14-positive group. One of these groups contained 18 (42%) of the tumours positive for CK14. This subgroup had significantly shorter overall survival than other three CK14-positive groups. These figures suggested the basal phenotype may not always warrant a poor prognosis and also

showed the heterogeneity within this particular set of tumours. This data is not an anomaly and heterogeneity of prognosis has also been recorded in the work of Sotiriou *et al.*,²⁶ Rakha *et al.*,²⁹ Laakso *et al.*,⁶¹ Jumppanen *et al.*⁶² and Reis-Filho *et al.*⁶³

Recently, Fulford *et al.*⁵⁹ performed an analysis on 443 grade III invasive ductal carcinomas with extended clinical follow. Cytokeratin 14 was used to identify the “basal” cohort (88 cases, 20%). Unlike many pan-grade studies, this work focused on grade III tumours only. They showed that over the first 5 years of follow-up, disease free survival and overall survival were similar for “basal” and “non-basal” tumours. However, the subsequent course for “basal” cancers was better than the “non-basal” grade III cancers, data that is contrary to the “basals are bad” conclusion of the expression profiling experiments. The data of Fulford *et al.*⁵⁹ are not inconsistent with the literature. All the recurrences in the “basal” group were in the first 5 years and indeed the prognosis of “basal” cancers appears to be bad in the early follow-up period. However, if the “basal” cancers do not relapse within the initial 5 years, they appear to have a relatively good prognosis subsequently. This also ties in to their observation that distant recurrence, in particular metastases to the brain, is more common in “basal” breast cancers than other grade III carcinomas,^{18 59} leading to death in the shorter time frame. The data support the observation by Jones *et al.*¹⁶ that there are

more than one subgroups of “basal/myoepithelial” cancers, one exhibiting early relapse and an aggressive clinical course and the other, despite the aggressive pathology, remaining relapse free. The challenge is now to develop parameters that will help identify these subgroups at the time of primary diagnosis.

There are no obvious pathological parameters that appears to predict for these subgroups, although researchers have suggested separation into “basal” versus “myoepithelial”²⁹ (tumours expressing basal cytokeratins versus tumours expressing muscle related proteins) and “basal” versus “basolumental”⁶¹ (tumours expressing HMW cytokeratins in a uniform versus a partial fashion). Fulford *et al*⁵⁹ also reported that the pattern of cytokeratin 14 staining was prognostically significant; tumours exhibiting a diffuse pattern of staining having a better prognosis (fig 1E,F).

THERAPEUTIC CONSIDERATIONS

“Basal” breast cancers are generally high grade, node negative and ER, PgR and HER2 negative. This phenotype generates interesting therapeutic dilemmas, especially with the knowledge that a proportion will have early relapse and death from metastatic disease. Anti-oestrogens and trastuzumab (Herceptin) are not an option for such patients, and currently chemotherapy and radiotherapy for systemic and local control remains the mainstay. However, the choice of chemotherapy remains unclear.⁶⁴

There is very little data of the effectiveness of chemotherapy in “basal” breast cancers. These tumours have a phenotype that contains many features associated with a good response to neoadjuvant therapy,⁶⁵ ie, high grade, ER negative, highly proliferative. It is interesting therefore that a prospective study by Rouzier *et al*⁶⁶ showed a 45% complete response to neoadjuvant paclitaxel and doxorubicin in “basal” cancers. In contrast, Banerjee *et al*,⁶⁷ in small series from a single institution, found that adjuvant anthracycline based chemotherapy was not as effective in “basal” breast cancers as non-basal cancers. This data has to be interpreted cautiously as it was a retrospective study with small numbers; a recent study by Diallo-Danebrock and colleagues suggested that basal like cancers do indeed do better with adjuvant chemotherapy.⁶⁸ The two studies are not directly comparable as the latter was carried out in patients who were node positive, and it is clear from the literature that basal tumours have a propensity to be node negative.

“Basal” breast cancers are associated with over-expression of EGFR. Anti-EGFR therapies such as cetuximab and gefitinib have been successful in other tumour types⁶⁹; however, currently there is little data to support their use in breast cancer. Clinical trials are currently underway to assess their efficacy.

Due to the overlap between the BRCA1 related and “basal” cancers (referred to as BRCAness),⁷⁰ it is logical to envisage that treatment options for BRCA1 related cancers may be effective in “basal” cancers. The DNA repair defects seen in BRCA associated cancers leads to sensitivity to cross-linking agents such as mitomycin-C and platinum based drugs. Again, there is little data to support the use in “basal” breast cancers, but investigations are ongoing to assess efficacy.

More recently, an elegant alternative has been suggested for BRCA1 tumours. In cells, single strand DNA breaks are repaired by a base excision system containing the enzyme PARP1 (poly(ADP-ribose) polymerase). It has been shown that in cells lacking BRCA1, inhibition of PARP1 sends the cells into apoptosis. PARP1 inhibitors could therefore be a novel treatment option for BRCA1 related and perhaps “basal” like cancers.^{71–72} The studies are in the very early stage of evaluation but hold much promise for the future.

Take-home messages

- Basal-like cancers are a subset with distinct morphology.
- They are often but not invariably triple negative (ER–, PgR– and HER2–).
- They have a distinct genomic, expression and protein profile.
- The subset is heterogeneous with respect to behaviour, not all being uniformly of poor prognosis.
- Currently, therapy is same as for other high grade cancers but new options are under investigation.

SUMMARY AND CONCLUSIONS

“Basal” cancers are a subgroup of breast cancers with a distinctive morphology and immunophenotype, and although not in the current WHO classification system for breast cancer,⁷³ they are very likely to be in the near future. Our recommendation would be that pathologist attempts to identify this subtype in clinical practice using a combination of morphology (high grade, high mitotic counts, medullary like, central scar/necrosis, spindle cell and squamous differentiation) and immunophenotype (ER, PgR and HER2 negative). The “basal” phenotype can then be confirmed using one or more of CK14, CK5/6 or EGFR. These tumours are more common in young women; in this situation, the clinical colleagues can be alerted about the possibility of familial predisposition.

Although specific therapeutic options are not currently available, it is only by recording and cataloguing the heterogeneous group that we will be in a position to define appropriate treatment options in the future.

ACKNOWLEDGEMENTS

We are grateful to Dr Peter Simpson, Dr Pria Pakkiri, Dr Olivier Ramuz and Dr Kelvin Lim for discussion and critical comments. Dr Leonard Da Silva is a clinical research fellow funded by The Ludwig Institute for Cancer Research.

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Competing interests: None declared.

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