Topical Review

Basal cell carcinoma: a dermatopathological and molecular biological update

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Summary

The ideal classification of basal cell carcinoma (BCC) should be able to identify subtypes which correlate with clinical behaviour and treatment requirements. Unfortunately, however, such a classification has yet to be defined. In the interim, the currently most favoured classification is one based predominantly on histological growth pattern. This classification contributes to the useful concept of low- and high-risk histological subtypes of BCC. The latter are characterized by an increased probability of subclinical extension and/or incomplete excision and/or aggressive local invasive behaviour and/or local recurrence. The Royal College of Pathologists has published a minimum dataset for the histopathological reporting of BCC and this has been written to be compatible with the British Association of Dermatologists’ management guidelines. Growth patterns to be reported include nodular, superficial, infiltrative/morphoeic and micronodular types, together with differentiation when of severely atypical or malignant squamous type (basosquamous carcinoma). Deep and peripheral excision margins will be reported to be either involved or clear. The latter will include a comment of a clearance of less than 1 mm for close margins and a measured distance in whole millimetres for other excisions. Clinical assessment and histology remain the ‘gold standard’ for evaluating BCC and cancers in general. However, in the postgenomic era emphasis is changing from the gathering and archiving of genomic data to its analysis and use in guiding clinical practice. In this context, a current goal is to define cancer phenotype in terms of molecular abnormalities and use this as a new gold standard. One way to assess whether this goal is being achieved for BCC is to determine whether our knowledge of its molecular pathology has any relevance to the minimum dataset for histological reporting. Knowledge of BCC molecular pathology has been fuelled by the recent discovery that deregulation of the Hedgehog (Hh) signalling pathway, a key player in embryonic patterning, appears to be fundamental to tumour growth. But despite accrual of a large amount of data concerning Hh pathway molecular alterations in neoplasia, little is known about the functional consequences of these changes in BCC, how they lead to tumour development, or how they relate to non-Hh pathway alterations such as TP53 mutation. Recent work suggests that the cellular localization of β-catenin gives a degree of credence to the growth pattern classification of BCC. Furthermore, it is possible that β-catenin may have a pathogenetic role in the invasive behaviour of BCC. This review draws on current evidence to discuss these issues and assess whether they are relevant to the minimum dataset.

Key words: basal cell carcinoma, classification, dataset, molecular biology
The dermatopathology of basal cell carcinoma

Classification

The ideal histopathological classification of basal cell carcinoma (BCC) should be based on subtypes that correlate with clinical behaviour and treatment requirements. In addition, the classification should be easy to use and reproducible.

To date, two main approaches to the classification of BCC have been suggested. One is based on histopathological growth pattern and the other on histological differentiation. Unfortunately, no universally agreed classification exists to date; it is generally regarded that classification based on growth pattern has the greatest biological significance.

Sexton et al.1 extended the earlier work of Sloan2 and suggested a classification that identified nodular, superficial, infiltrative, morphoeic, micronodular and mixed patterns. Support for this approach has been provided by Rippey3 and it is this classification that has been adopted by the Royal College of Pathologists (RCPath) in its minimum dataset for the reporting of common skin cancers (Table 1).4

Classification by growth pattern is also useful in developing the concept of low- and high-risk histological subtypes of BCC. The high-risk subtype is characterized by a variable combination of potential clinical problems, including an increased probability of subclinical extension and/or incomplete excision and/or aggressive local invasive behaviour and/or local recurrence.

Nodular BCC is the main low-risk subtype and the term has been used in the RCPath dataset to incorporate numerous histological variants. In particular, this includes nodulocystic BCC which displays spaces due to mucin accumulation and also those BCCs with follicular differentiation and keratin cysts. Although rare, fibroepithelioma of Pinkus appears to be a recognizable low-risk subtype with a distinct growth pattern.

High-risk BCCs include superficial, infiltrative/morphoeic and micronodular subtypes. Superficial BCC (previously and inappropriately termed multicentric or multifocal) is associated with an increased risk of recurrence, due to an increased tendency of incomplete primary excision. Infiltrative/morphoeic BCCs can be associated with aggressive local invasive behaviour, with a tendency to increased recurrence. Both are characterized by irregular groups of tumour cells with a spiky appearance. The morphoeic variant of infiltrative BCC is characterized by the presence of stromal fibrosis.

In micronodular BCC, the tumour islands are round and by definition less than 0.15 mm in diameter.5 It is characterized by an increased tendency for subclinical extension.6

In general, the type of histological differentiation in a BCC has gained less support as a basis for classification. At least 20 subtypes have been described4 and the significance of squamous differentiation in BCCs is still debatable.

Specifically, there is no universally agreed usage for the term basosquamous carcinoma. For example, the term is used to describe collision tumours of basal and squamous cell carcinomas, keratotic and follicular BCCs and also the controversial entity of metatypical BCC. Despite this confusing situation, however, there is evidence that BCCs associated with moderate/severe squamous atypia or squamous malignancy are associated with a higher incidence of recurrence and metastatic spread.7 A minor amount of squamous atypia is not unusual in BCCs showing follicular differentiation and this does not appear to be biologically significant.

In addition, several general difficulties confront histopathologists reporting BCCs, using any type of classification: (i) many BCCs have more than one growth pattern and published works have varied on the percentage necessary to designate the presence of a specific subtype; (ii) the importance of the pattern present at the invading edge of the tumour has received only limited research consideration; (iii) although there is an ongoing study in the U.K., the reporting reproducibility of the subtypes has been inadequately investigated; and (iv) only adequate trimming protocols can ensure that the minimum clearance margins in the sections examined are truly representative of the specimen.

Accordingly, until more research evidence is available, the RCPath has recommended that the growth pattern subtypes should be reported when any subtype constitutes 50% or more of the lesion and/or when a high-risk subtype constitutes a component of the invading edge or is present next to the resection margin. A standardized trimming protocol has also been recommended.

Minimum dataset for histopathology reporting

The RCPath’s minimum dataset for common skin cancers is designed to achieve reporting consistency,
with respect to both terminology and content. Its usage is intended to aid patient management, by the provision of the necessary information to assist clinical decision-making. It also provides prognostic information for the patient and clinician and provides feedback to the clinician with respect to the quality of treatment. The dataset also constitutes the histopathological section of the National Skin Cancer Dataset for the National Health Service Information Authority.

The RCPath was pleased to receive advice from the British Association of Dermatologists (BAD) and the dataset was specifically designed to provide adequate information for those dermatologists who are fully implementing the BAD guidelines for the management of BCC. In particular, this relates to the provision of prognostic information that also allows the clinician to select the most appropriate form of treatment.

With reference to excision margins, the terms adequate/inadequate and complete/incomplete have been avoided in the dataset, as their usage can be subjective and imply inappropriate clinicopathological insight (Table 1).

Careful consideration was also given as to whether peripheral and deep clearance should be measured quantitatively. The BAD advised that it is clinically necessary to know whether the peripheral and deep margins are clear or involved and when a BCC is close to the nearest margin. Unfortunately, however, there is no agreed definition of close and publications to date have suggested variable measurements ranging from less than 0.31 mm to less than 0.84 mm or even simply less than 1 high power field. Furthermore, these figures vary according to the growth pattern of BCC and the size of cell nests.

For that reason, the most straightforward approach appeared to be measurement of peripheral and deep clearance to the nearest whole millimetre. In view of the limited evidence base to define a close margin to the nearest one or two decimal points below 1 mm, when applicable, a comment of ‘less than 1 mm’ has been recommended. The recommendation in the dataset to provide a specifically measured distance (to the nearest whole millimetre) with wider clearance has, however, proved to be a contentious issue. It must be emphasized, however, that the RCPath’s datasets are not written in tablets of stone and are updated regularly in the light of new information and opinion. For example, if the BAD collectively advised that there is no requirement routinely to provide specific clearance measurements of more than 1 mm, then this aspect could be removed from the next edition of the dataset.

The time has now come for BCC to be given the clinical and histopathological respect it deserves. Old-fashioned histopathology reports such as ‘Basal cell carcinoma. Excision complete’ are no longer acceptable and indeed most pathologists and clinicians now appreciate the gross inadequacy of this type of report.

Although BCC is an extremely common malignancy and there are personnel shortages in histopathology, it would be unfortunate if the standard of dataset reporting was lowered merely to accommodate short-term problems. For the histopathologist, having ‘signed-up’ to report close excisions of below 1 mm, little additional time is taken to provide a measurement of greater than 1 mm. Interestingly, similar reservations occurred in the reporting of measured circumferential excisions in colonic cancer. Initially, histopathologists were reluctant to undertake the extra task and surgeons were less than keen to audit their surgical practice against this measurement. Circumferential excision measurement is, however, now fully established as a vital standard to audit clinical practice in the treatment of colonic cancer.

The molecular biology of basal cell carcinoma

Histological assessment remains the cornerstone of a tumour’s analysis and this is especially true for BCC, where our understanding of its cellular defects remain limited. However, a key goal for cancer biologists is to relate the cancer phenotype to a list of defining molecular principles. Recently, it was found that many BCCs have mutations in the Patched1 (PTCH1) gene, which plays a crucial role in embryonic patternning and is a member of the Hedgehog (Hh) signalling pathway. This part of the review will describe what has been learned about the Hh pathway’s role in BCC, its relationship to other genetic changes, and finally assess whether this has any relevance to histological reporting.
Hedgehog pathway biochemistry

The Hh gene, encoding a secreted ligand, was originally identified in screens for mutations that disrupt segment polarity in the fruit fly, Drosophila.13 The gene has three vertebrate homologues, of which sonic Hh (SHH) is the most widely expressed.14,15 The Hh ligand binds to a membrane receptor, PTCH1.16,17 On binding, PTCH1-mediated inhibition of the trans-membrane protein, Smoothened (SMOH), is relieved allowing the latter to transduce a signal into the cell.18–20 This results in altered activity of the Gli family of transcription factors. In essence, the binding of Hh to PTCH1 switches on Gli-mediated expression of pathway target genes such as PTCH1 and Gli1.

The Hedgehog pathway and basal cell carcinoma

Gorlin’s syndrome is a rare condition, featuring the development of multiple BCCs.21,22 Germline mutations in these patients were found in the PTCH1 gene along with somatic alterations in the second allele in the ensuing tumours.23,24 Subsequently, somatic alterations of both PTCH1 alleles were found in sporadic BCCs.25 In addition, mutations in SHH,26 and SMOH19,27,28 were identified in BCC, and Gli1 gene amplification was found in glioma.29 The common effect of these genetic alterations is constitutive Hh pathway target gene induction. This is supported by the finding of PTCH1 overexpression in all 36 BCCs in one study.30 This suggests that Hh pathway deregulation is likely to be a very early cellular event in BCC. The finding of loss of heterozygosity at chromosome 9q22.3, the PTCH1 locus, in both small and large tumours31 and clonal loss in multiple independently analysed nests from superficial BCC32 provides additional support for this proposal. However, the most striking evidence that Hh pathway deregulation is an early event in BCC formation comes from in vivo transgenic model systems. In these studies, the Hh pathway was deregulated in epidermal cells of transgenic mice33–35 and in transgenic, reconstituted, human skin.36 Tumours that were indistinguishable from BCC developed within weeks, in the absence of mutation in other genes such as HRAS and TP53.34 Current evidence therefore suggests that Hh pathway deregulation alone can rapidly generate BCC directly from normal keratinocytes. This may explain why, in contrast to melanoma and squamous cell carcinoma, BCC has no apparent precursor lesion.

How does Hedgehog pathway deregulation drive tumour growth?

Although a large body of evidence now links Hh pathway deregulation to BCC genesis, very little is known about how this cellular defect exerts its tumorigenic effect. Because pathway target gene induction appears to be crucial, it will be important to determine the nature of these genes in keratinocytes. Those found to be upregulated in BCC include PTCH1, Gli1, WNT2B/WNT5A37 and hedgehog-interacting protein,38 but it is unknown whether their expression is causally related to Hh pathway deregulation. Other putative target genes continue to be identified. Recently, for example, cyclin D and E were identified in Drosophila.39 There is also evidence that Hh pathway activation can mediate a direct cytoplasmic effect, upstream of target gene activation, by altering the cellular distribution of cyclin B1 with a resultant positive effect on cell cycle progression.40

Many functional effects have been ascribed to Hh pathway activation and these may be relevant to its role in BCC. One possibility is that activation of the Hh pathway leads to expansion of stem cells numbers, as demonstrated in Drosophila ovary.41 Mouse cerebellar granule cell precursors42,43 and pluripotent human haematopoietic cells.44 This might explain the histological appearance of BCC as a proliferation of undifferentiated, basaloid keratinocytes. In this scenario, one could speculate that BCC is a hybrid between an abnormality of stem cell fate/patterning and a neoplastic proliferation. The pathway’s role in hair growth may be particularly relevant to BCC. Mouse gene knockout studies have established that a lack of Hh pathway signalling results in arrested hair follicle development,45,46 while anti-SHH antibodies inhibit anagen hair growth.47 In contrast, transient pathway activation stimulates anagen.48 Taken together, these data show that the Hh pathway is important in embryonic hair development and postnatal hair cycling. This may explain why the transgenic models described above lead to the formation not only of BCC, but also of follicular tumours such as trichoblastomas, trichoepitheliomas and cylindromas. This implies that BCC is part of a spectrum of follicular tumours, albeit the least differentiated. The basis upon which different tumours occur is not known, although one study showed that trichoepithelioma shows a greater degree of Hh pathway target gene induction than BCC49 and cylindromas are associated with mutation of a recently identified gene, CYLD1, that is distinct from the Hh pathway.50
How does molecular analysis impact on the minimum dataset for histopathological reporting?

The minimum dataset for BCC emphasizes low-risk and high-risk BCC histology. Does our increased understanding of molecular pathology inform that classification? In particular, how can a single molecular abnormality, Hh pathway deregulation, account for different histological patterns and their disparate behaviours? It seems likely that other factors modify the effect of Hh pathway deregulation. For example, the increased frequency of superficial BCC on the trunk might indicate that body site is a disease modifier. Alternatively, genetic abnormalities in non-Hh pathway genes, occurring over time, and frequently related to the damaging effects of ultraviolet radiation, might underpin these differences. Such temporal progression from one histological type to the next is supported by the age distribution of the tumours, with superficial BCCs occurring on average at 56.8 years, nodular BCC, 63.9 years, and infiltrative BCC, at 66.0 years. However, there is no hard evidence to support such tumour progression. For instance, while a reduction in E-cadherin and bcl-2 proteins is seen in the aggressive, infiltrative type of BCC, immunohistochemistry for p53 protein shows conflicting results. Additionally, TP53 mutations, present in up to 50% of BCCs, are seen in infiltrative, nodular and superficial tumours. Furthermore, the precise temporal relationship between the acquisition of Hh pathway deregulation and TP53 alteration remains unclear. Mutations involving the ras family are an uncommon and inconsistent finding in BCC and show no preference for histological type. The relationship between Hh pathway alterations and syndromes with inherited BCC predisposition, distinct from Gorlin’s syndrome, is unclear. These include Bazex syndrome, with putative dominant inheritance linked to Xq24–q27, and Rombo syndrome, whose gene has not been mapped. In contrast to earlier work by Boonchai et al., El-Bahrawy et al. have recently shown that the immunohistological localization of β-catenin gives a degree of credence to the growth pattern classification of BCC used in the minimum dataset. In particular, all subtypes had their own characteristic pattern of membranous, cytoplasmic and nuclear staining. Nuclear localization was most notably seen at the invasive margins of infiltrative, nodular and superficial BCCs, suggesting a possible role for β-catenin in tumour invasion. β-catenin is a key player in the Wnt/Wg signalling pathway, directly mediating downstream events through transactivation of transcription factors of the lymphocyte enhancer factor (Lef)/T-cell factor (Tcf) family. One of the target genes is the gene encoding the matrix-degrading metalloproteinase, matrilysin and this could be one mechanism by which β-catenin may play a role in tumour invasion. Interestingly, although micronodular BCC emerged as a distinct subtype, there was no nuclear localization of β-catenin. This does indicate, however, that alternative molecular mechanisms to any disturbance of the E-cadherin/catenin complex are also operative in the invasive behaviour of BCC. It is possible that the nuclear β-catenin expression may reflect the highest Hh pathway activity and thereby highest Gli1 activity and increased Wnt expression, as proposed previously and illustrated in Figure 1.

Therefore, while there is strong evidence that Hh pathway deregulation is a key event in BCC tumorigenesis, the relationship of this and other molecular defects to different histological patterns and behaviour remains unclear.

One situation where molecular biology might assist histological assessment is when BCC is part of a
might be a viable treatment option. In the future, crucial in determining whether pathway suppression Hh pathway mutations. These questions will prove expression. However, the level of target gene expression or reverse transcription–polymerase detection of increased target gene expression could appear to be present in virtually all BCCs, molecular techniques may help to resolve differential diagnosis. Because Hh pathway deregulation appears to be present in virtually all BCCs, detection of increased target gene expression could serve as a useful tumour marker by using in situ hybridization or reverse transcription–polymerase chain reaction to assay PTCH1 or Gli1 mRNA. Unfortunately, in the differentiation of BCC from trichoepithelioma, both may show increased target gene expression. However, the level of target gene expression may help to differentiate between superficial BCC and actinic keratosis and in assessing whether a hair follicle or BCC is present at an excision margin.

In conclusion, our understanding of BCC has been fuelled by the discovery that Hh pathway deregulation is an essential cellular abnormality. However, many questions remain unanswered, such as the nature of the Hh pathway target genes, and the influence of non-Hh pathway mutations. These questions will prove crucial in determining whether pathway suppression might be a viable treatment option. In the future, molecular techniques may help to resolve differential diagnostic dilemmas associated with BCC, but, at present, molecular biology fails to explain key clinico-pathological considerations.

References


