Uveal melanoma: cell cycles and survival

The ability to predict the fate of patients after the removal of a neoplasm has remained a perpetual goal for pathologists. To accomplish this, the modern pathologist not only seeks to distinguish between benign and malignant tumours, but also to anticipate which of those frankly malignant neoplasms will pose the greatest threat to survival. To this end, various methods of classifying or staging a wide variety of tumours have been devised.

In 1931 Callender¹ devised a histological classification for uveal melanomas. Accordingly, tumours were divided into six groups: spindle cell A, spindle cell B, fascicular, mixed, epithelioid, and necrotic; based upon their cellular morphology or histological architecture. Subsequent studies have demonstrated the value of this classification in predicting patient survival. Paul *et al*² in a retrospective study of 2652 melanomas of the ciliary body and choroid found that patients with spindle cell A tumours had a 15 year survival rate of over 80%; in sharp contrast with patients with epithelioid tumours where the overall survival was less than 30%. Although the Callender classification was universally accepted and has been used in its original form for many years, certain problems have emerged which have led to its eventual modification. A more recent classification divides melanocytic uveal tumours into three groups; spindle cell naevi, spindle cell melanomas, and mixed cell melanomas.^{3,4} While this modification is an undoubted improvement, a fundamental problem still remains – namely, it is subjective and relies entirely upon the judgment of the pathologist.

Several studies (albeit using the original classification) have emphasised the problem of interobserver error. MaLean *et al*⁵ reviewed a series of 212 melanomas and found that they subsequently reclassified 12% of the tumours. Char *et al*⁶ found that two ocular pathologists affiliated with the same university classified uveal melanomas differently 17% of the time! These discrepancies are hardly surprising: if a pathologist finds a single epithelioid cell in a field of spindle cells should he classify that tumour as of mixed cell type? Probably not. But what if the tumour contains five or 10 or 50 epithelioid cells per field? At what point should he make that crucial step and assign it mixed cell status? In an attempt to circumvent these problems ocular pathologists have investigated the role of quantitative techniques in assessing the metastatic potential of uveal melanomas. These efforts have focused on the morphology of the cell nucleus; for, while both cell shape and abundance of cytoplasm are factors in determining cell type, it is the appearance of the nuclei which is probably crucial in influencing the metastatic potential of the tumour. Gamel *et al*⁷ developed a computerised technique which randomly selected cells in a standard histopathological section; various measurements could then be made on the nucleus and its nucleoli. A number of these nuclear variables were found to correlate with patient survival. Further studies⁸ confirmed that the standard deviation of nucleolar area (SDNA), especially if combined with the measurement of the largest tumour dimension (LTD),⁹ was the most accurate quantitative cytological determinant of patient survival. Unfortunately, although measurement of the SDNA appeared to be a valid and reproducible method of assessing malignant potential, it was time consuming and required elaborate specially designed equipment. More recently, a simpler technique which determines the mean of the largest nucleoli (MLN) has been developed.¹⁰ While this method

is undoubtedly less time consuming, it still requires an experienced technician to obtain the measurements.

Gross structural abnormalities of the nucleus reflect, at least in part, aberrations of the cell karyotype. The normal human somatic cell contains 46 chromosomes (23 pairs) and is referred to as diploid. A cell with fewer or more than 46 chromosomes is described as aneuploid. Karyotypic abnormalities are a well recognised feature of human cancers. The loss or gain of a chromosome may alter the amount of specific cell products and, if these are crucial in controlling cell division and differentiation, may lead to unrestrained proliferation. Furthermore, genetic abnormalities may lead to increasing karyotypic instability and a higher rate of spontaneous mutation which, in turn, will permit the more rapid progression to a highly malignant phenotype.^{11,12} Recently, investigators have analysed the karyotypes of uveal melanomas and found non-random chromosome abnormalities.¹³⁻¹⁵ While karyotypic analysis may provide us with clues as to the initiating events in the development of neoplasms, it is extremely time consuming and requires fresh tumour tissue; for these reasons it is unlikely that it could be used routinely as a method of assessing prognosis. In contrast, flow cytometry is an automated method of quantifying the cellular DNA content of a tumour. In essence, a suspension of tumour cells is obtained and stained with fluorescent dye which will bind directly to DNA. This labelling is stoichiometric – that is, the degree of staining is directly proportional to the amount of DNA present. The suspension is then passed through a flow cytometer which can rapidly measure the amount of fluorescence, and hence the amount of DNA in an individual cell. The analysis of several thousand cells provides information about the ploidy and rate of cellular proliferation of the tumour. Studies in other human tumours using this technique have linked the presence of aneuploidy with poor prognosis.¹⁶ Several investigators¹⁷⁻²⁰ have utilised the technique of flow cytometry in an attempt to predict survival in patients suffering from uveal melanomas with mixed and conflicting results. Meecham and Char¹⁷ found that the presence and degree of aneuploidy was associated with reduced survival. Other authors have failed to demonstrate an association.¹⁸

In this issue of the *BJO* Hodge *et al* report their findings (p 521) using this technique on paraffin embedded material obtained from 45 posterior uveal melanomas. They found that the relative risk of death was approximately 3.5 times greater in patients with aneuploid tumours when compared with diploid ones. They also found that an increasing percentage of cells in S phase (an indicator of cell proliferation) was associated with reduced survival. They could not, however, demonstrate a relation between survival time and an increasing degree of aneuploidy. Their results are in broad agreement with the previously published results and suggest that, although this technique may have a role to play in determining survival in these patients, further work is required to confirm this. To date, with one exception,¹⁹ all studies have been retrospective using archival paraffin embedded tissue. Hodge *et al* emphasise the need for a standardised prospective study of flow cytometry using fresh tumour tissue: I endorse this proposal.

The search for accurate pathological determinants of survival in cases of uveal melanoma is a necessary one. There are, however, limitations: histological variation and genotypic aberrations do not predict the response of the host, if

any, to the tumour; nor do they anticipate the impact of treatment on the eventual outcome. It is conceivable that timely intervention may prevent dissemination of a tumour, despite an apparently aggressive phenotype. Ultimately, a foolproof method of predicting survival may be unattainable. None the less the quest continues.

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