THE ASHTON LECTURE

UVEAL MELANOMA: THE PAST, THE PRESENT AND THE FUTURE

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Uveal melanomas are the most common primary intraocular neoplasm in adults.[1] In spite of this, most general ophthalmologists will probably encounter only a handful of them during their career. Mindful of their malignant potential, we assume that prompt treatment is vital, and that destruction of the primary tumour will influence survival. This rationale is not new, indeed Fuchs in 1882 commented that patients with uveal sarcoma (melanoma) almost inevitably die from their disease and that enucleation was the treatment of choice.[2] Since then ophthalmologists, often in collaboration with other disciplines, have devised alternative methods of treatment which avoid removal of the eye and, it is hoped, retain useful vision. Broadly speaking, these techniques involve radiotherapy, photocoagulation, surgical resection or a combination of these modalities.[3]

Radiotherapy was probably first used in 1930 by Moore,[4] who implanted radon seeds into a melanoma in a patient's only useful eye. Later, Stallard[5] refined the use of brachytherapy for these tumours by using cobalt-60 episcleral plaques. In 1964 Lommatzch introduced ruthenium-106 episcleral plaques; since then, brachytherapy has become an increasingly popular form of treatment. Other radioactive isotopes have been evaluated and, of these, iodine-125 has gained a wide acceptance.[6–8] More recently, charged particle external beam irradiation, using either protons or helium ions, has been used to treat uveal melanomas.[9–11] We have recently evaluated the administration of external beam irradiation using the Leksell gamma knife.[12]

In 1952, Meyer-Schwickerath[13] first used photocoagulation to treat choroidal melanomas. Whilst this technique initially gained popularity, the high incidence of local complications, including tumour recurrence, limits its application as a primary treatment.[14] Increasingly, its use is restricted to that of an adjuvant to other therapies.

Surgical resection of both ciliary body and choroidal melanomas has become an accepted technique in the last few decades. Although various surgical approaches have been advocated,[15,16] the technique of partial lamellar sclero-uvectomy originally pioneered by Foulds[17] has been adopted by a number of ocular oncologists.

Whether these techniques which avoid removing the globe as a primary procedure adversely affect survival is uncertain. Indeed a large-scale prospective study which compares brachytherapy with enucleation is currently in progress.[18] Whilst they reduce patient morbidity, it is doubtful whether any of these conservative techniques actually improves survival. It is a depressing fact, known to all those who regularly attend to such patients, that many, perhaps up to 50%[1,19] of all those treated, will ultimately die from their disease.

Why do patients who have apparently effective irradiation of the primary tumour die from tumour-related disease? The simple answer is, of course, that they die from distant metastases. But why should this occur? It is well recognised that the incidence of overt metastases at the time of presentation is very low: most published studies report a frequency of only 2% or 3%.[20,21] Yet within 2–3 years of treatment, a significant number of patients develop and succumb to metastatic disease. These observations have led some clinicians to suggest that removal of the primary tumour by enucleation may actually promote the dissemination of malignant cells and adversely affect survival. They argue that surgical

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manipulation of the globe at the time of enucleation increases the intraocular pressure and, thus, physically squeezes tumour cells into the circulation.[22,23] If correct, this argument could, equally, be applied to other treatment modalities which involve surgical intervention, including local resection, radioactive plaques and insertion of localising rings prior to charged particle external beam irradiation. Others have challenged the validity of this hypothesis and suggested that prompt treatment is essential if the patient's chance of survival is to be improved.[24] Although the arguments which support this hypothesis may, at first, seem persuasive, they considerably underestimate the complexity of the metastasic process. A malignant cell, capable of producing a distant metastasis, must traverse several natural barriers, evade the host's immune system and, then, have the capacity to undergo unrestrained replication and to induce neovascularisation in this new site. The metastatic process is probably extremely inefficient, with perhaps only a tiny percentage of tumour cells which enter the circulation possessing the necessary attributes to successfully establish a distant colony of tumour cells.

The answer to the question of why patients still die despite adequate treatment of the primary tumour seems inescapable: they die because micrometastases have been established before the clinician has had a chance to institute any treatment. These deposits, which are undetectable at presentation, or at the time of primary treatment, remain dormant for a variable period of time, which may be many years,[25] before enlarging and, perhaps, undergoing further tumour dissemination, to produce detectable lesions which will lead ultimately to the death of the patient. This scenario is almost certainly observed in a variety of other tumours and is not unique to uveal melanomas. Why tumour cells remain dormant, what factors may suppress their growth, and what stimuli may eventually lead to their proliferation are unclear.

Although up to half the patients treated for a uveal melanoma ultimately die from their disease, it is obvious that approximately half of the affected patients survive. Perhaps it is pertinent to ask why these patients survive. In theory, it would seem that three different explanations can be proffered. Firstly, it is probable that when a uveal melanocyte undergoes malignant transformation to produce unrestrained local proliferation, the resulting tumour, although locally invasive, may not be capable of metastasising. In time, perhaps as a result of clonal selection, a cohort of cells will be produced which possess the necessary phenotypic properties to produce metastases. The point in time when the tumour becomes metastatically competent will be, to a large extent, a unique feature of that individual tumour. Thus, if at the time of presentation the tumour, although possessing clinical features suggestive of malignancy, was incapable of producing metastases, effective treatment at this point would be curative. Secondly, the tumour may have been capable of producing metastases prior to treatment, but the patient's immune system was successful in irradicating them. Finally, micrometastases may develop prior to treatment but remain dormant, the patient then subsequently dying of an unrelated disease. In this last situation the treatment would appear to have been effective despite the presence of occult residual disease. If these arguments are correct then it is apparent that the only way to improve survival in patients with uveal melanoma is to identify those patients who have micrometastases at the time of presentation or, failing this, at least identify those with the greatest risk of micrometastases, and devise treatment strategies which will promote destruction of the metastatic cells.

THE METASTATIC POTENTIAL OF UVEAL MELANOMA

It would seem at present that we do not have the necessary techniques to identify occult micrometastases. However, whilst it may not be possible to identify every patient with occult disease, it may be possible to recognise those patients who are at greatest risk of developing metastases. Currently, a number of clinical and pathological determinants are recognised predictors of survival (and by inference metastatic potential). Clinical features which may influence prognosis include: age of the patient, tumour size, anatomical location, integrity of Bruch's membrane, pigmentation, and secondary glaucoma.[26–31]

Tumour size is probably the most important single clinical parameter in determining prognosis. In a recent meta-analysis of 5 year mortality rates following enucleation, Diener-West *et al.*[30] found mortality rates of 16% for small tumours, 32% for medium tumours and 53% for large tumours.

The role of tumour location in determining prognosis is both fascinating and controversial. Several studies have shown that involvement of the ciliary body and/or the location of the tumour's anterior border being anterior to the equator is a poor prognostic sign.[28,29,32,33] It has been suggested that anterior tumour location is an independent predictor of increased mortality;[29] other studies have suggested that the association is primarily due to ciliary body tumours being larger and possessing a more malignant cytology.[34] As if to complicate matters further, Glynn *et al.*[35] found that ciliary body involvement had greater significance in predicting those patients who died in 2 years of treatment, in contrast to those who died subsequently.

Callender,[36] in 1931, was the first to classify uveal melanomas according to histological morphology and cell type. He divided uveal melanomas into six groups: spindle A, spindle B, epithelioid, mixed, fascicular and necrotic. The spindle A, spindle B and epithelioid groups contain tumours composed predominantly of that cell type. Mixed tumours are composed of a variable mixture of spindle and epithelioid cells. Fascicular tumours are composed of spindle cell (either A and/or B) arranged in ribbons or fascicles. Necrotic tumours, as the name implies, are composed almost entirely of necrotic tissue, where the cell type cannot be established. Several studies have demonstrated the usefulness of this classification in predicting survival.[37] Paul *et al.*,[37] in 1962, studied the impact on survival of histological cell type in 2652 cases of uveal melanoma. They found 15 year survival rates of 81% for spindle A tumours, 73% for spindle B or fascicular, 41% for mixed or necrotic and only 28% for epithelioid tumours. Recently, this classification has been modified and uveal melanocytic lesions are now divided into three groups: spindle cell naevi, spindle cell melanomas and mixed cell tumours.[38] These changes reflect the difficulties encountered by ocular pathologists when trying to classify certain tumours. Most spindle cell melanomas are composed of a variable mixture of A and B cells, and trying to apportion them to one category is often impossible. Similarly, the point at which a tumour containing predominantly epithelioid cells should be classified as a pure epithelioid tumour is difficult, if not impossible, to define.

Although the Callender classification has proved a useful predictor of survival, it suffers from one major limitation: it is subjective and relies on observer interpretation of the specimen. As a result, significant rates of disagreement between pathologists have been reported when attempting to classify uveal melanomas.[39,40] In general, epithelioid cells have larger nuclei and nucleoli (which are often multiple) than spindle cells and these differences in themselves have been shown to predict survival.[41] Based on these observations, Gamel and McLean[42–45] devised an objective computerised morphometric analysis system which computed the standard deviation of the nucleolar area (SDNA) and its inverse (ISDNA). This latter variable, especially when coupled with the largest tumour diameter, is a valuable indicator of survival. In addition it has been shown that there is a correlation between reduced survival time and a low ISDNA score.[46] Unfortunately, these estimates of nuclear pleomorphism are time-consuming, require expensive equipment and, most importantly, require a significant technical input. The techniques are thus limited to large laboratories or research facilities. More recently, a simpler technique which measures the maximum diameter of the ten largest nucleoli (MTLN) using a manually operated micrometer has been found to compare favourably with other histopathological parameters of malignancy, including the SDNA.[47,48]

There is, of course, an obvious problem with all histopathological methods of assessing malignant potential: they require tumour tissue, obtained either from enucleation or local resection specimens. The metastatic potential of tumours in patients who are treated by other methods, where tumour tissue is unavailable, cannot be evaluated by histological means. One solution would be to obtain tumour tissue by fine needle aspiration biopsy. This technique can provide the histopathologist with enough tissue to perform a cytological analysis which will permit the differentiation between uveal melanoma and lesions which may simulate them.[39,49] Unfortunately, there is a poor correlation between the tumour cell type determined by fine needle aspirate and that found at subsequent histological examination of the enucleated tumour.[39] Moreover, attempts to perform morphometric techniques such as SDNA or ISDNA on fine needle aspirates have met with little success.[50,51] Char *et al.*[51] concluded that these measurements were affected by cell type, specimen processing and investigator experience.

Investigators have attempted to circumvent this problem by developing clinical, non-invasive methods of predicting the tumour cell type or morphology. Coleman *et al.*[52] examined 46 patients with uveal melanoma prior to enucleation with a computerised diagnostic ultrasound system. The tumours were characterised by ultrasonically measured dimensions and power spectrum analysis. Following enucleation the histopathological morphology and cell type were quantified and compared with the ultrasonic measurements. Significant correlations were found between the power spectrum analysis and the histological characteristics. Although this technique may provide improved *in vivo* prognostic indicators for uveal melanomas, it does not use conventional ultrasonography and is, at present, not widely available.

Recently, an association has been found between clinical estimates of tumour vascularity in cutaneous melanomas and values obtained from subsequent histopathological examination.[53] Moreover, there appears to be a correlation between the vascular density of tumour vessels in histological preparations of cutaneous melanoma and survival.[53,54] As a result of these studies Folberg *et al.*[55] examined vascular patterns in histological sections of uveal melanomas. They found that nine morphological patterns of tumour vessels could be identified by examining tissue sections stained with fluorescein-conjugated *Ulex europaeus* I (a marker of vascular endothelium)

using laser confocal microscopy or when viewed after staining with a modified periodic acid–Schiff reaction viewed with a green narrow bandpass filter. They found that the presence of closed vascular loops was associated with poor survival. Subsequent studies have confirmed that vascular loops, particularly when they form vascular networks (which are composed of three back-to-back closed loops) are associated with death from metastatic disease.[56–58] It is hoped that a clinical, *in vivo* method of detecting these vascular patterns will be developed in the future.

So far, I have discussed some of the known clinical and pathological determinants of survival. Useful as these indices may be, they still do not answer the fundamental question: why do some tumours metastasise and others apparently do not. Why, for example, does a cell with an epithelioid morphology apparently have a greater ability to metastasise than a cell with a spindle morphology? The immediate response to this question is that epithelioid cells resemble the primitive neural crest melanocyte and, as such, represent a relatively undifferentiated cell, whereas the spindle cell, in resembling the mature uveal melanocyte, reflects a greater degree of differentiation. It is well recognised in many other cancers that the degree of differentiation has an important impact on survival. However, the question can still be asked: Why do undifferentiated tumours have a poor prognosis?

In attempting to answer this question we are, in effect, addressing the fundamental questions regarding the factors that may cause a cell to undergo malignant transformation. There is now considerable evidence that mutations within the cell genome cause cancer.[59] The observation that chromosome abnormalities are observed in many types of cancer[59] supports this hypothesis. Chronic myeloid leukaemia (CML) was the first malignancy in which a reproducible chromosomal abnormality was described. The leukaemic cells from almost all patients with CML contain a unique small chromosome, called the Philadelphia chromosome (Ph chromosome). In 1973, Rowley[60] suggested that the Ph chromosome resulted from a reciprocal translocation between chromosomes 22 and 9. The exact location of the break has been identified, and there is now unequivocal evidence that the rearrangement is a reciprocal translocation and that it alters the structure of the *abl* oncogene.[59] Further studies indicate that at least 93% of patients with acute non-lymphocytic leukaemia have marked chromosomal abnormalities and that many of these have a prognostic significance.[61] A number of specific, non-random chromosome abnormalities have now been identified in a variety of malignancies since the detection of the Ph chromosome. Indeed, one of the most important and best studied is in the field of ophthalmology: the mutation on the long arm of chromosome 13 in retinoblastoma. Other well-recognised chromosome abnormalities include deletion of the long arm of chromosome 5 (5q) in colon carcinoma, deletion of the short arm of chromosome 11 in Wilms' tumour, the loss of the short arm of chromosome 1 in neuroblastoma and the loss of the short arm of chromosome 3 in both small cell carcinoma of the lung and renal cell carcinoma.[59]

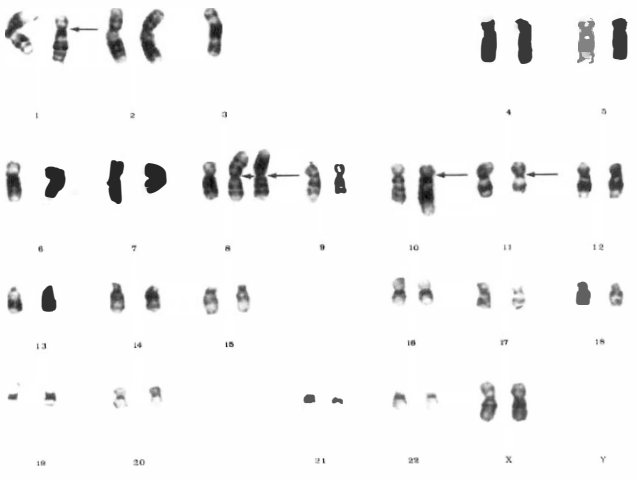

Fig. 1 *A karyotype of a ciliary body melanoma. Note the loss of chromosome 3 and additional iso 8 chromosome.*

Several years ago my co-workers and I elected to examine the karyotypes of uveal melanomas, in an attempt to gain a greater understanding of the basic biology of these tumours. In essence, this technique involves obtaining fresh tumour cells from either local resection or enucleation specimens and growing them in short-term tissue culture for a minimum of 1 week. Cell divisions are then harvested by the addition of Colcemid. Chromosome preparations are made on clean, cold, wet slides, which are subsequently G-banded with trypsin and Leishman's stain. In an initial study published in 1990 we reported the cytogenetic findings in six posterior uveal melanomas. Abnormalities of chromosome 1 and 6 were noted in two and four cases respectively and, interestingly, three ciliary body tumours demonstrated both monosomy 3 and iso8q (Fig. 1).[62] These results were in agreement with the previously published literature on the cytogenetics of uveal tumours. Rey *et al.*,[63] in 1985, reported the karyotypic features of a brain metastasis from a uveal melanoma. They found anomalies of both chromosomes 6 and 8. Griffin *et al.*[64] in 1988, observed a single abnormality (trisomy 6p) in a solitary uveal melanoma. Prescher *et al.*[65] reported their findings in 14 cases of uveal melanoma and found an increased dosage of chromosome 8 in eight patients (57%) and monosomy 3 in six patients (43%). Subsequent cytogenetic studies have confirmed abnormalities of chromosomes 3, 6 and 8[66–69] together with other, less frequently observed abnormalities including anoma-

lies of chromosomes 9 and 11.[66,69–73] More recently, the technique of comparative genomic hybridisation has been used to detect chromosomal deletions or additions in uveal melanomas.[74,75] These studies have provided confirmation of the abnormalities first isolated by karyotypic analysis.

One striking feature of the karyotypic abnormalities in uveal melanomas is the localisation of tumours with anomalies of chromosomes 3 and to the ciliary body.[76,77] Indeed it has been suggested that the relatively poor prognosis observed in patients with ciliary body tumours is attributable to these karyotypic abnormalities.[77] In a subsequent study using both standard karyotypic analysis and comparative genomic hybridisation, Prescheri *et al.*[78] found by univariate analysis that monosomy 3 was the most significant predictor of survival.

We have recently reported the results of an extensive study on the cytogenetics of 42 melanomas using conventional karyotypic techniques.[79] In ten cases this was supplemented by dual colour fluorescent *in situ* hybridisation (FISH) to confirm the abnormalities of chromosomes 3 and 8 (Fig. 2). The majority of the tumours were either medium or large in size with a median value of 15.8 mm (range 8–25.2 mm) for the largest tumour diameter (LTD). Similarly, the majority of the tumours had significant numbers of epithelioid cells, with 31 (73.8%) being classified as mixed or pure epithelioid cell. Twenty-three tumours (54%) had one or more additional copies of the long arm of chromosome 8 (either an additional chromosome 8 or iso8q) and, of these, 14 had two or more copies. Monosomy 3 was found in 21 (50%) of the tumours examined. Other abnormalities included deletion of chromosome 1p in 12 patients (28.6%), deletion of 6q in 13 patients (31%), trisomy 6p in 8 patients (19%), trisomy 21 in 8 patients (19%) and abnormalities of chromsome 11 in 9 patients (21.4%). This study confirmed the strong association between involvement of the ciliary body and abnormalities of chromosomes 3 and 8. Of the 23 tumours with additional copies of 8q, 19 involved ciliary body ($p = 0.0002$, Pearson chi-square). Furthermore, 19 of the 21 tumours exhibiting monosomy 3 also involved the ciliary body ($p = 0.001$).

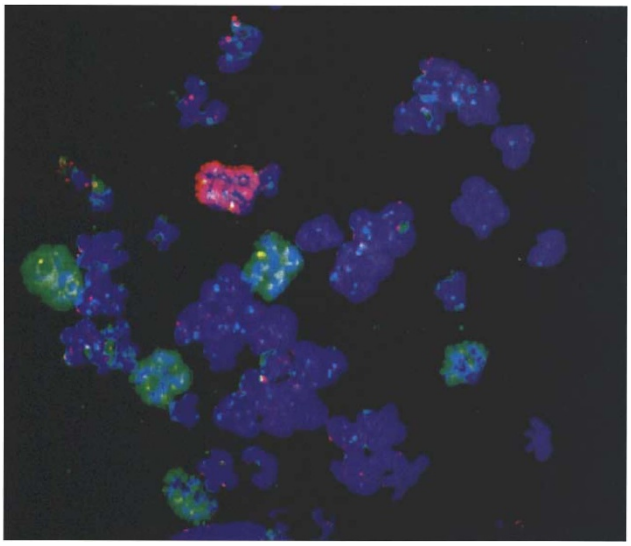

Fig. 2. *A double colour fluorescent* in situ *hybridisation (FISH) preparation of a ciliary body melanoma. There is one copy of chromosome 3 (red) and five copies of chromosome 8 (green).*

In the second part of this study we investigated the possible relationship between karyotypic abnormalities and survival. Kaplan–Meier survival curves were constructed for the various karyotypic anomalies, together with curves for parameters which have previously been reported to be of prognostic significance. These include age, location, tumour size and cell type. Of these parameters, only monosomy 3 ($p = 0.0007$, log rank Kaplan–Meier), additional 8q ($p = 0.0027$) and tumour location ($p = 0.003$) achieved statistical significance. Furthermore, when the number of extra copies of 8q was compared with survival, it was found that patients with two or more copies had a significantly worse prognosis ($p = 0.0001$). Although interesting, these results must be viewed with some caution; the median duration of follow-up was only 30.5 months (range 8–96 months). Moreover, there is an inherent bias in this group of patients, with the majority of the tumours being either medium or large in size and most containing significant numbers of epithelioid cells. It is probably this bias which, at least in part, explains why these traditional factors fail to achieve significance. In effect, the cytogenetic abberations appear to be a predictor of survival in an already high-risk group. This, of course, only serves to increase their power as predictors of survival.

Karyotypic analysis is a demanding and time-consuming technique and as such is unlikely to be of value in the routine evaluation of uveal tumours. It does, however, provide us with some insight into the genomic events which may control development and subsequent dissemination of these tumours. I have already mentioned the importance a genetic mutation in the development of tumours. There is increasing evidence to suggest that these mutations affect the normal function of genes that control and regulate cell proliferation. Such mutations have been observed in two functionally and genetically different classes of genes in human tumours: (i) dominantly acting oncogenes which are thought to be responsible for cell growth, and overexpression of which will result in tumorigenesis; and (ii) suppressor genes (or anti-oncogenes) which normally restrict or inhibit cell proliferation, and loss of function of which leads to the development of a tumour. However, it is unlikely that this is a single event and mathematical

models constructed to explain the increase in cancer with age suggest that at least five to seven mutations are necessary for malignant transformation of a normal cell.[80] Investigations into the genetic events leading to the development of carcinoma of the colon, a tumour particularly suited to study because of its slow development and progression through histologically distinct benign and malignant phases of growth, suggest that a progressive series of events leads to the ultimate development of a metastatically competent tumour.[81]

The consistent nature of the karyotypic abnormalities in uveal melanomas points to important sites in the genome where mutations may have occurred. Monosomy 3 is a consistent abnormality occurring in 50% of the tumours we have studied. This would suggest presence of a suppressor gene on chromosome 3 which, when lost, contributes to the development of the tumour. Evaluation of the tumour karyotype by conventional cytogenetics can only identify gross features such as the loss of an entire chromosome or a significant fragment of a chromosome; they cannot, of course, detect loss of individual genes. The loss of small fragments of genetic material which may contain specific genes can be detected by techniques developed in the evolving field of molecular biology. We have used the technique of restriction-fragment-length polymorphism (RFLP) analysis to examine the loss of genetic material on chromosome 3 in greater detail.[82] This technique, as the name implies, uses restriction enzymes which recognise specific sequences in DNA and will split it at that site. If a mutation has occurred at that site the restriction enzyme will fail to recognise the sequence and cleavage will not occur. Thus mutations will produce changes in the length of the restriction fragments. These polymorphisms are common, and can be used to determine alteration at specific sites on a chromosome. We studied 20 tumours with markers for both arms of chromosome 3 and found loss of heterozygosity (LOH) in 60% of them. When compared with cytogenetic studies which were available on some of the tumours, we found LOH could occur in the presence of an apparently normal chromosome 3. This suggests that the incidence of loss of genetic material located on chromosome 3 is greater than simple cytogenetic studies would suggest. This in turn adds support to the hypothesis that loss of a suppressor gene on chromosome 3 is involved with the tumorigenesis of uveal melanomas.

It is interesting to note that cytogenetic abnormalities of chromosome 3 occur in other solid tumours, notably small cell carcinoma of the lung and renal carcinoma. Small cell carcinoma of the lung appears to have a consistent deletion in a portion of the short arm of chromosome 3 – de13p (p14p23) – and renal tumours also demonstrate deletion of 3p. It is tempting to speculate that loss of one (or more) suppressor genes located on the short arm of chromosome 3 may be a common link in the development of these tumours.

The abnormalities of chromosome 8 are, in some respects, even more intriguing: the increased amounts of genetic material which results from additional copies of chromosome 8, or more specifically from 8q, would suggest the presence of a promoter oncogene located on this chromosome. The increase in gene dosage which results from these cytogenetic aberrations may lead to increased expression of this gene. The observation that metastatic potential may be directly linked to the number of extra copies of 8q suggests that an oncogene (or oncogenes) located in this region may have a crucial role in the metastatic spread of these tumours. Laboratory studies using tumour models have implicated the role of certain oncogenes in metastasis. Non-metastatic tumour cell lines and some immortalised fibroblast cell lines can be converted into metastatic lines by transfection with activated oncogenes, in particular the H-*ras* oncogene.[83,84] Moreover, mutant forms of *myc* and p53 can induce cells that are already tumorigenic to become metastatic.[59] The fact that c-*myc* an oncogene involved in cellular proliferation, is located on chromosome 8q24.1, led us to investigate its expression in uveal melanomas.

We examined 24 uveal melanomas by immunohistochemistry, using two monoclonal antibodies raised against a mid-sequence portion of the c-*myc* product.[85] This was compared with other putative prognostic factors including tumour size and cell type. c-*myc* expression was also compared with the proliferation index, an indicator of the rate of cell proliferation, as determined by flow cytometry. No correlation was found between c-*myc* expression and size or cell type, but a strong correlation was found with rate of proliferation ($p = 0.0001$, Wilcoxon signed rank test). A comparison between c-*myc* expression and the cytogenetics was possible in only nine of the tumours. Again, no relationship was found between the expression and extra copies of chromosome 8q. The results suggest that increased c-*myc* expression is related to the rate of cell turnover, rather than the initiator of this abnormal cell cycling. Further studies are required to evaluate the role of this and, perhaps more importantly, other oncogenes located on chromosome 8q.

TUMOUR IMMUNOLOGY: THE POTENTIAL FOR THERAPEUTIC INTERVENTION

So far I have concentrated on those factors responsible for dictating survival in patients with uveal melanomas; and whilst these studies may provide us with important information concerning the mechan-

isms responsible for promoting tumour dissemination, they do not, at least at present, provide us with a method of improving survival. If we accept the hypothesis that tumour dissemination has already occurred by the time we initiate traditional therapy, then prevention of further dissemination (that is dissemination for existing metastases) combined with destruction of the micrometastases would appear to be the only method of effecting a permanent cure. Unfortunately, to date, we have no way of detecting occult micrometastases, although with improved methods of identifying high-risk patients, we can infer that such deposits are probably present. In these patients some form of adjuvant therapy would appear to be ethically justified, provided any side effects are within acceptable limits. One theoretically attractive method of addressing the problem of micrometastases would be to use some form of adjuvant immunotherapy.

The precise role of the immune system in the control of cancer has historically been one of continuing controversy and, over the years its popularity as a possible therapeutic weapon has waxed and waned. However, recent advances in the field of tumour immunology suggest that, at last, its true potential may be realised. It has been known some years that some patients have clonally expanded tumour-specific $CD8^+$ T cells which may be found in the peripheral blood and the primary tumour site.[86] Much of the current interest in this field relates to the recognition of specific tumour-associated antigens which may be recognised by these cytotoxic T cells. In 1991, Boon and his associates[87] identified a gene which encoded a tumour antigen on melanoma cells that was recognised by cytotoxic T cells. Subsequently, they were able to isolate a family of genes from human cutaneous melanoma that encode for antigens recognised by cytotoxic T cells. These genes, which are now called *MAGE* genes (Melanoma Antigen Genes), are present in all normal cells but remain transcriptionally silent (with the exception of testis and placenta) until neoplastic transformation occurs. In all, 12 closely related *MAGE* genes have been located to the q terminal region of the X chromosome.[88] Although originally identified in cutaneous melanomas, six (*MAGE* –1, –2, –3, –4, –6, –12) have been found to be expressed in a variety of other tumours including carcinomas of the breast,[89,90] lung,[91] bladder[92] and stomach.[93] The initiation of a tumour-specific cytotoxic T cell response requires that MAGE antigens are presented by HLA class 1 molecules on the surface of the tumour cells. This is an important prerequisite, for the tumour cell must express the correct HLA class 1 molecule in order for the tumour-specific antigen to be presented to the T cell.

The potential clinical significance of these observations relates to the fact that short peptides derived from the processing of *MAGE*-1 and *MAGE*-3 gene products are capable of inducing a cytotoxic T cell response and are, therefore, potential candidates for a tumour-specific peptide vaccine immunotherapy. Pilot studies using MAGE peptide vaccines in the treatment of cutaneous melanomas are currently being undertaken. Thus, it would seem that MAGE peptide vaccine therapy may be of potential value in the treatment of uveal melanomas; provided, of course, that these antigens are expressed by these tumours. In collaboration with the Ludwig Institutes for Cancer Studies in Brussels and Switzerland, we conducted a study into the expression of *MAGE*-genes (*MAGE* –1, –2, –3, –4) in primary and metastatic uveal melanomas using reverse transcription followed by polymerase chain reaction (RT-PCR) amplification.[94] Unfortunately, we failed to find any significant *MAGE* gene expression in any of the 27 primary tumours studied. Furthermore, *MAGE* gene expression was detected only in 2 of the 26 metastatic tumours analysed. Regrettably, our data suggest that, unlike cutaneous melanomas, uveal melanomas may not be suitable candidates for *MAGE* peptide immunotherapy.

Although tumour-specific antigens (e.g. MAGE antigens) represent an ideal target for immunotherapy because they are not expressed in the majority of (if not all) normal tissues, tumour-associated antigens may offer an alternative, if less specific, target. There is a group of peptides processed from the melanocyte lineage-specific proteins – tyrosinase, gp100 and Melan-A/MART-1 – which are present in cutaneous melanoma and have been shown to induce cytotoxic T cells.[95–98] Whilst the possibility exists that treatment with peptides derived from these differentiation antigens of the melanocyte may induce a reaction against normal melanocytes, vaccines derived from these epitopes could be of potential value in treating patients with disseminated disease. Again, pilot studies using melanoma-associated peptides are currently being undertaken in patients with disseminated cutaneous melanoma. We have examined the expression of the melanocytic lineage peptides (tryosinase, gp100 and Melan-A/MART-1) in uveal melanomas and found that, unlike the MAGE antigens, they are expressed at high levels.[94] On the basis of these results, we intend to proceed to a pilot study using melanocyte-associated peptide vaccines in patients with disseminated uveal melanoma.

CONCLUSIONS

Our preoccupation with developing methods of eradicating the primary tumour in uveal melanoma, in the absence of any viable alternatives, is born of

necessity. Many patients in whom tumour dissemination has already occurred prior to presentation, destruction of the primary tumour probably represents nothing more than 'closing the stable door after the horse has bolted'. The hope for the future is that by acquiring a greater understanding of the basic biology of this tumour, we shall be able to develop new forms of treatment which will not only be effective against the primary tumour, but will also address the problem of disseminated disease.

In pursuing the study of these tumours I have been privileged to work with many collaborators, in particular Bob Rees, Andy Parsons, Sheila McNeil and Karen Sisley. To them I should like to express my gratitude. I should also like to thank all my colleagues who continue to refer me these patients. Finally, I wish to acknowledge the considerable financial support for this work provided by the Yorkshire Cancer Research Campaign, the Special Trustees for the Former United Sheffield Hospitals and the Medical Research Council.

Key words: Uveal melanoma, Cytogenetics, Oncogenes, Immunology.

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