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CAMBRIDGE OPHTHALMOLOGICAL SYMPOSIUM

Molecular pathology of uveal melanoma

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Abstract

Like other cancers, uveal melanomas (UM) are characterised by an uncontrolled, clonal, cellular proliferation, occurring as a result of numerous genetic, and epigenetic aberrations. Signalling pathways known to be disrupted in UM include: (1) the retinoblastoma pathway, probably as a result of cyclin D1 overexpression; p53 signalling, possibly as a consequence of MDM2 overexpression; and the P13K/AKT and mitogen-activated protein kinase/extracellular signal-related kinase pathway pathways that are disturbed as a result of PTEN and GNAQ/11 mutations, respectively. Characteristic chromosomal abnormalities are common and include 6p gain, associated with a good prognosis, as well as 1p loss, 3 loss, and 8q gain, which correlate with high mortality. These are identified by techniques such as fluorescence *in situ* hybridisation, comparative genomic hybridisation, microsatellite analysis, multiplex ligation-dependent probe amplification, and single-nucleotide polymorphisms. UM can also be categorised by their gene expression profiles as class 1 or class 2, the latter correlating with poor survival, as do *BRCA1-associated* protein-1 (BAP1) inactivating mutations. Genetic testing of UM has enhanced prognostication, especially when results are integrated with histological and clinical data. The identification of abnormal signalling pathways, genes and proteins in UM opens the way for target-based therapies, improving prospects for conserving vision and prolonging life.
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Introduction

Uveal melanoma (UM) is the most common primary adult intraocular cancer. More than 90% involve the choroid, the remainder being confined to iris and ciliary body. UM affects approximately six individuals per million per year in the United Kingdom, with the age at diagnosis peaking at 50–60 years. It is markedly different to cutaneous melanoma in its clinical and molecular genetic features. Concerning predisposition to UM, rare reports of families with an excess of UM cases have been published.[1–3] Recent evidence suggests that patients with a cancer susceptibility may have higher frequencies of UM compared with the normal population.[4] Other risk factors are congenital ocular melanocytosis, melanocytoma, and neurofibromatosis.

There is a wide range of therapeutic options for the treatment of primary UM. These include various forms of radiotherapy, surgical resection, and phototherapy.[5] The 5-year local-tumour control rates in most specialised treatment centres exceed 90%. Despite this, almost 50% of patients with UM will develop disseminated disease, predominantly in the liver, but also in the lungs (24% of patients) and bone (16%).[6] Early surgical removal of metastases has improved patient survival in some cases;[7,8] however, in general, the prognosis of UM patients with metastatic disease is currently poor because of the lack of effective chemotherapeutic agents.

Intense efforts have been made in the last decades to understand the molecular genetics involved in the development and the progression of UM, to recognise those that are likely to metastasise, and to identify signalling pathways and possible 'druggable' molecules in the neoplastic melanocytes, which can be targeted using systemic therapies. Some of the early genetic events causing disruption of the cell cycle and apoptotic control in uveal melanocytes have been determined, as well as

those leading to their malignant transformation and metastasis promotion.

This review summarises the current insights into the molecular mechanisms underlying UM pathogenesis, prognostic tests, and potential therapeutic strategies.

The hallmarks of cancer

Cancer is defined as an uncontrolled, clonal proliferation of cells, which progressively acquire most if not all of the six 'hallmarks' of neoplasia, as defined by Hanahan and Weinberg in 2000, and then updated in 2011.[9,10] These hallmarks are considered to be absolute biological prerequisites of neoplastic cells for their survival and proliferation capacity at the primary site, and for their ability to invade and metastasise (Table 1). These features are acquired by a multistep process and include: (1) insensitivity to anti-growth signals; (2) self-sufficiency in growth signals; (3) avoiding apoptosis; (4) limitless replicative potential; (5) sustained angiogenesis; and (6) tissue invasion and metastasis.

These six core characteristics of the malignant cell are acquired in different tumour types via distinct mechanisms and at various times during the course of multistep tumourigenesis. An increasing body of evidence suggests that two additional 'emerging' hallmarks of cancer and two 'enabling characteristics' are involved in the pathogenesis of some and perhaps all cancers. These were added in the revised article of Hanahan and Weinberg in 2011, and include: (1) deregulating cellular energetics; (2) avoidance of immune destruction; (3) tumour-promoting inflammation and (4) genome instability and mutation (Table 1). As these capabilities have yet to be generalised to all tumour types and yet to be fully validated, they are still considered to be provisional.[10]

How do these hallmarks of cancer relate to UM?

UM is considered to be a cancer arising from uveal melanocytes. The precursors of melanocytes are non-pigmented melanoblasts derived from the neural crest, which bypass natural tissue barriers and basement membranes of the eye when migrating during embryogenesis. As with processes occurring in the skin, the melanoblasts mature into melanocytes within the uvea, and/or give rise to melanocytic stem cells, which maintain the ocular melanocytic 'system'. Although it is known that melanocytic stem cells reside in the hair bulge in the skin,[60] the location of uveal melanocytic stem cells is still unknown.

It is hypothesised that various genetic and epigenetic alterations occur along the 'melanoblast–melanocyte–naevus–UM' pathway, resulting in their malignant

transformation and propensity to spread. It is unclear whether the 'melanoma-initiating' or 'cancer stem-like' cells, recently shown to be present in UM cell lines,[61] are derived directly from ocular melanocyte progenitors or from more mature melanocytes that have de-differentiated. Recent analysis of gene expression data of UM would suggest that de-differentiation indeed does occur during UM development, but this requires further investigation.[11,39] It is also unclear whether the naevus stage is a prerequisite in UM development: it has been estimated that <1 in 8000 naevi undergo malignant transformation to form UM.[62] Histologically, it is exceptionally rare for a residual naevus to be evident adjacent to or within a choroidal melanoma, supporting this observation.

Despite this large gap in our knowledge regarding initial UM development, it has been demonstrated by several groups that most (if not all) of the 'hallmarks of cancer' can be applied to UM pathogenesis (Table 1). The genetic and epigenetic events involved in UM development and dissemination enable malignant uveal melanocytes to proliferate and survive autonomously. These events include: mutation or amplification of proto-oncogenes; inactivating mutations or deletions of tumour (and metastasis) suppressor genes; and chromosomal aberrations (Tables 1, 2 and 3).[11] Some of these genetic alterations are considered to occur in the early stages of UM development; others, at later stages (ie, prior to or at haematogeneous dissemination). It has been proposed that the genetic developmental pathway of UM bifurcates at an early stage to result in two very distinct genetic signatures: (1) disomy 3 with chromosome 6p gain and class 1 gene expression profile, and (2) monosomy 3 with class 2 molecular signature and a high metastatic propensity.[40,78,80,81] Later genetic events in UM development are suggested to be increasing aneuploidy and alterations in chromosome 8 (eg, gains in 8q and loss of 8p; see below for further details).

Although this dichotomous model is helpful in 'classifying' UM into two risk groups with respect to the development of metastasis, it essentially discounts any concept of clonal heterogeneity within UM, which has been demonstrated by several groups using differing methods.[82–86] Furthermore, it does not fit well with the concept of clonal evolution.[87,88] Recent evidence does indeed suggest that this model may be too simple, and that different clones of malignant melanocytes may evolve and co-exist within UM, with some having the potential to override or dominate others.[79] This has been observed in longitudinal studies using next-generation sequencing in paired primary and metastatic carcinomas,[89] and warrants further investigation using such techniques in UM.

Table 1 The classic and emerging 'hallmarks of cancer' and their application to UM

Hallmarks of cancer	Example of gene/mechanism affected	Mechanism(s) in UM	References
Classical hallmarks			
1. Insensitivity to anti-growth signals	Loss of cell cycle inhibitor, such as retinoblastoma (Rb) suppressor	The retinoblastoma tumour suppressor pathway is disrupted in most UM either through: hyperphosphorylation of Rb; elevated expression of cyclin D1; or through methylation and inactivation of the *INK4A* gene	11–16
2. Self-sufficiency in growth signals	Gain of cell cycle stimulator—activation of pathways	The PI3K–AKT prosurvival pathway is constitutively activated in UM. LOH of the PTEN locus occurs in 76% of UM	17–21
		The RAF/MEK/ERK pathway is constitutively activated: activating mutations in *GNAQ or GNA11* occur in >80% of UM and can activate the RAF/MEK/ERK pathway	11,22–28
3. Avoid apoptosis	p53 pathway alterations	The p53 pathway is functionally blocked by its inhibitor MDM2	13–15,29,30
	BCL-2	Defects in the Bcl2 pathway in UM contribute to apoptosis resistance	11,14,29
	PTEN downregulation	The PI3K–AKT prosurvival pathway is constitutively activated in UM to avoid apoptosis	19,20
	Produce insulin-like growth factor (IGF-1) survival factors	IGF1R is often upregulated and can activate the PI3K–AKT pathway	31,32
4. Limitless replicative potential	Turn on telomerase	Upregulated telomerase activity in UM	33,34
5. Sustained angiogenesis	Production of vascular endothelial growth factor (VEGF) inducer either by the tumour cells or by accompanying inflammatory cells	Upregulated expression of VEGF in UM; association with macrophage densities in UM	35–37
		Increased expression of IGF-1 and IGF-1R in UM	32,38
		Raised levels of hypoxia-inducible factor 1 alpha	39–41
6. Tissue invasion and metastasis	Activation of E-cadherin	Upregulation of E-cadherin and Wnt/beta-catenin signalling pathways	42,43
		Downregulation of the helix-loop-helix inhibitor ID2	39,40
		Increased expression of matrix metalloproteinases (MMPs) and downregulation of their tissue inhibitors (TIMPs) by UM	44–47
		ALCAM expression	48
		NOTCH pathway activation	49
		Biallelic methylation of EFS	50
Emerging hallmarks			
1. Avoid immune destruction	Reduced tumour cell immunogenicity	Expression of PD-L1 by UM regulates T-cell function by suppressing IL-2 production and impairing T-cell function	51
	Downregulation of HLA class 1 and 2 expression	Downregulation of HLA class 1 expression on UM cells	52,53
	T-cell exhaustion		51
2. Deregulating cellular energetics	Upregulating glucose transporters, for example, GLUT1, resulting in substantial increases in glucose import into cytoplasm. Hypoxia response of tumours acts by upregulating glucose transporters and multiple enzymes of the glycolytic pathway	Indirect evidence through the increased levels of the HIF1α and HIF2α transcription factors in UM	39–41
Enabling characteristics			
1. Genome instability and mutation		See Tables 2 and 3	See Tables 2 and 3
2. Tumour-promoting inflammation	Lymphocytes Macrophages Dendritic cells	Varying densities of tumour infiltrating lymphocytes and macrophages; both associated with worse prognoses	54–59

Table 2 Known genetic and epigenetic alterations in UM

Proto-oncogenes affected	Mechanism	Chromosome	Frequency (%)	References
NRAS	Mutation	1p13	a	63–66
BRAF	Mutation	7q34	a	67,68
NSB1	Amplification	8q21	50	69
MYC	Amplification	8q24	43	70
DDEF1 (ASAP1)	Amplification	8q24	50	71
GNAQ/GNA11	Mutation	9p21	>80	25–27
CCND1	Amplification	11q13	65	12,14–16
MDM2	Amplification	12q15	65	13–15,29,30
BCL-2	Amplification	18q21	>95	11,14,29
Tumour-suppressor genes				
LZTS1	Deletion	1p13	—	72
CDKN2A-sporadic	Deletion, mutation	9p21	a	1–3
CDKN2A-familial	Deletion, mutation	9p21	a	1–3
PTEN	Deletion, mutation	10q23	15	20
BAP1	Inactivating mutation		0[b]–84%	31
Epigenetic alterations				
CDKN2A	Hypermethylation	9p21	4–33%	1–3,48,73,74
RASSF1	Hypermethylation	3p21.3	13–70%	28,75
hTERT	Hypermethylation		52%	74
MicroRNA alterations				
let-7b	Overexpression	NA	c	76,77
miR18a	Overexpression	NA	c	76,77
miR-199a	Overexpression	NA	c	76,77
miR495	Overexpression	NA	c	76,77
miR549	Overexpression	NA	c	76,77

Abbreviation: NA, not applicable.
[a] Rare.
[b] Data from Lake et al, unpublished results.
[c] Not documented.

Table 3 Most common chromosomal aberrations in UM

Chromosome	Frequency[a]
1p loss	28–34%
1q gain	24%
3 loss	50–61%
6p gain	28–54%
6q loss	35–37%
8p loss	17–28%
8q gain	36–63%

[a] Combined data of references Hoglund et al[78] and Damato et al.[79]

In the following paragraphs, the major molecular pathway defects and the most common chromosomal alterations in UM development will be summarised.

Molecular pathway defects in primary UM

In most UM, the retinoblastoma (Rb) and p53 pathways are functionally inhibited, although actual mutations in the RB1 and TP53 genes are rare.[11–13,29,30,90] The Rb protein is constitutively hyperphosphorylated and functionally inactivated in most UM, probably as a result of cyclin D1 overexpression in about 65% of cases.[12,14,15] (Figure 1; Table 1) The role of CDKN2A promoter hypermethylation, as an additional mechanism of Rb inactivation, is controversial as the frequency of hypermethylation of this gene varies between 4 and 33% of UM.[12,14–16,48,74] Increased cyclin D1 protein expression has been associated with larger tumour basal diameter, epithelioid cell type, and poor prognosis.[15] The p53 pathway is inhibited downstream to p53 in many UM,[30] and this may be a consequence of MDM2 overexpression, which is also common in UM and associated with a poor outcome.[13,15]

The PI3K/AKT pathway is constitutively activated in most UM[17] (Table 1). It has been demonstrated using immunohistochemistry that phosphorylated AKT correlates with poor prognosis in UM.[18] While a study of 9 UM cell lines did not observe mutations in PTEN,[19] a much larger study of 75 primary UM demonstrated loss of heterozygosity of the PTEN locus in 76% of tumours and actual mutations within the PTEN coding region in 11% of tumours.[20] PTEN inactivation is also associated
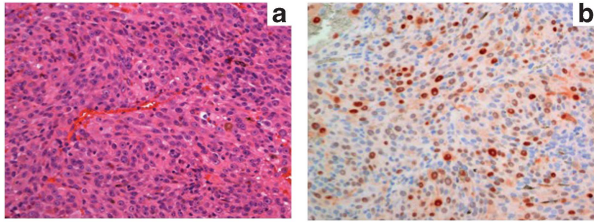
Figure 1 (a) Histological section of a UM of mixed cellularity (HE, ×20 magnification). (b) Immunostaining of the same tumour with clear nuclear cyclin D1 staining (APAAP, ×20 magnification).

with increased aneuploidy and decreased survival in UM.[20,21] Taken together, these findings implicate a role for PTEN in UM progression, indicating scope for further investigation in this area.

The mitogen-activated protein kinase/extracellular signal-related kinase pathway (MAPK/ERK) pathway is essential for mediating cell-cycle progression, and in several types of cancer mutations in this pathway result in it being constitutively activated, producing inappropriate and autonomous proliferation of neoplastic cells.[91] Most UM demonstrate constitutive activation of the MAPK pathway, suggesting the presence of upstream activating mutations.[11,22,23] However, a systematic interrogation of candidate oncogenes in the MAPK pathway undertaken by several groups was initially disappointing.[24] For example, mutations in KIT and the three RAS family members, which can activate the MAPK pathway, were shown to be exceedingly rare in UM.[23,63–66,92] Similarly, BRAF mutations, well described in cutaneous melanomas, have been reported in choroidal melanomas,[67] but are very rare.[22,23,65,68,93] Further, mutations in the other two members of the RAF family, ARAF and CRAF, are not found in UM.[24]

Guanine nucleotide-binding protein G(q) subunit (*GNAQ*, or G-alpha-q) and *GNA11* mutations

This puzzle concerning the MAPK pathway, and the mechanisms by which it was constitutively activated, persisted until the recent discovery of mutations in *GNAQ* in almost half of UM.[24,25] *GNAQ* is one of a subfamily of G protein α subunit encoding genes, comprising *GNAQ*, *GNA11*, *GNA14* and *GNA15/16*. The *GNAQ* mutation is somatically acquired, arising almost exclusively in exon 5 at codon 209 and resulting in substitution of the original glutamine at this point. There are at least five known variants, with GNAQ^{Q209L} or GNAQ^{Q209P} being the most frequent.[25] More recently, mutations of codon 209 were also discovered in *GNA11*, and both *GNAQ* and *GNA11* can also have mutations of exon 4 affecting codon 183.[26] Thus, in a recent study, over

80% of UM were found to have *GNAQ* or *GNA11* mutations affecting either Q209 or R183 in a mutually exclusive pattern.[26]

GNAQ and *GNA11* mutations are also found in uveal naevi and in most UM regardless of their tumour stage, chromosomal constellation or other outcome predictors (see below).[27] These mutations appear to be necessary but not sufficient for complete malignant transformation to melanoma.[25] These data suggest that *GNAQ* and *GNA11* mutations are early events in the molecular pathogenesis of UM. Therefore, although the timing of the alterations in the MAPK pathway during UM development have been clarified to some extent, the temporal sequences of those affecting the Rb, p53 and PI3K/AKT pathways remain to be determined.

Chromosomal alterations in primary UM

It has been known for almost 20 years that UM show specific chromosomal alterations, which are quite distinct from melanomas at other sites, particularly those of the skin (Table 3). The most striking abnormality in UM is the complete or partial loss of chromosome 3. Other common genetic abnormalities of UM include loss on 1p, 6q, 8, and 9p as well as gain on 1q, 6p, and 8q (Table 3). These alterations were initially identified by standard karyotypic analyses.[94–103] They have subsequently been confirmed by several groups using differing technologies, including: fluorescence *in situ* hybridisation (FISH);[104–109] comparative genomic hybridisation (CGH);[21,110–116] spectral karyotyping;[117,118] microsatellite analysis (MSA);[119–124] multiplex ligation-dependent probe amplification (MLPA),[79,125–127] and single-nucleotide polymorphisms (SNPs).[109,128–132]

The above-mentioned chromosomal alterations in primary UM are clinically relevant because of their correlation with the risk of metastatic death. Chromosome 3 loss is associated with a reduction of the 5-year survival probability from approximately 100% to <50%.[98,101] Similarly, chromosome 8 gains and loss of chromosome 1 significantly correlate with reduced survival.[79,104,108] Both chromosome 3 loss and polysomy 8q are also associated with other poor prognostic factors, including increasing tumour basal diameter, ciliary body involvement, presence of epithelioid cells, high mitotic count, and closed connective tissue loops.[79] Conversely, gains in chromosome 6p correlate with a good prognosis, suggesting this aberration has a functionally protective effect.[133]

Molecular techniques used for prognostication in primary UM

The dramatic finding of the adverse prognostic significance of chromosome 3 loss in UM was confirmed

by several groups, but was only translated from the 'bench to the bedside' by a very few ocular oncology centres initially for use in the clinical management and counselling of patients.[134] Since 1999, the cytogenetic status of UM cells has been assessed in consented UM patients undergoing either enucleation or local tumour resection at the Liverpool Ocular Oncology Centre using FISH.[108] As a result of low specificity with respect to detecting partial deletions of chromosome 3 (also associated with a poorer prognosis[79]), the Liverpool team, in late 2007, replaced FISH with a polymerase chain reaction-based technique, MLPA, which examines 38 loci across chromosomes 1p, 3, 6, and 8. This was done after validation of this technique using a cohort of UM with a median follow-up of over 6 years[125] and following comparative analysis with other techniques.[135] MLPA, or MSA in selected cases where limited DNA is generated for analysis, is currently provided on a routine basis to all UM patients requesting genetic tumour typing. Those receiving radiotherapy or phototherapy undergo a tumour biopsy. The costs are modest and covered by the UK National Health Service (NHS). It is important to note that the genetic testing is performed in an accredited molecular pathology laboratory, and is undertaken with both regular internal and external quality assessment in accordance with European and UK Genetic Testing Network guidelines (http://www.ukgtn.nhs.uk/gtn). This is also the case for the co-author (MZ) performing MLPA and MSA at the Institute of Human Genetics in Essen, Germany.[124] Consequently, the results of these tests can be considered as robust: they are certainly not 'investigational' or 'unethical', as has been unjustly suggested elsewhere.[136]

In the meantime, other ocular oncology centres are also offering molecular prognostication testing for their UM patients, using other techniques (Table 4), which are both DNA and RNA based. There are advantages and disadvantages to all methods (Table 4). The most commonly used tests are FISH, MLPA, MSA, SNP array (aSNP) and a PCR-based 12-gene assay based on gene expression profiling (GEP).[137] The latter technique divides UM into two 'classes' on the basis of an mRNA expression signature:[138] class 1 and class 2. Class 1 UM often show 6p and 8q gain.[40] Class 2 UM, tend to show more aneuploidy with 1p loss, 3 loss, 8p loss, and 8q gain. Class 2 UM are also strongly associated with inactivating mutations of 'BRCA1-associated protein-1' (BAP1), located at 3p21 (see below).[139] The GEP-based test has been patented (DecisionDx-UM; www.castlebiosciences.com/test_UM.html) and has received a considerable amount of publicity in the lay press, as it is claimed by Harbour and associates, its originators, to be superior to all other testing methods.[137,138] A recent study was performed to compare the prognostic accuracy of the GEP assay and monosomy 3, detected by a ''multi-SNP'' assay, which was designed by the authors as a research tool and still requires external validation as a clinical molecular test.[128,138] Although the GEP-based assay is marketed as a 'stand-alone' assay for prognostication, MLPA has been demonstrated to provide as accurate prognostication when considered with clinical and histomorphological features of UM. Consequently, the study by Onken et al, that assessed monosomy 3 alone, fails to compare the most robust approach to genetic prognostic testing available. In addition, preliminary studies comparing the GEP-based assay with MLPA-based prognostication incorporating clinical and histomorphological tumour features in formalin-fixed paraffin-embedded material, show high concordance between these two techniques, with respect to the tumours examined and in their correlations with predictors of metastasis (Coupland et al, unpublished data). It is also worth noting that survival data in the literature suggest that as many as 15% of class 1 UM metastasise, and these have belatedly been reclassified as class 1B (http://talkabouthealth.com/what-are-the-potential-results-from-the-decisiondx-um-gene-expression-profiling-assay). Furthermore, class 1 profiles are observed in some liver metastases, an observation that also needs further investigation.[129]

Finally, it is worth considering that molecular genetic diagnostics is a very rapidly advancing field with the costs of technologies plummeting at astounding rates, previously not experienced by the industry. Tests now considered to be 'state of the art' are therefore likely to have a short 'shelf life' and will be soon outdated by more accurate and less costly methods.

Molecular alterations proposed to be involved in UM metastasis

Aberrations of several genes and signalling pathways appear to promote dissemination of UM cells: these include LZTS1 (located on chromosome 8p22),[72] DDEF1 ('development and differentiation enhancing factor 1', also known as ASAP1, mapping to chromosome 8q24.21),[71] PTP4A3 ('protein tyrosine phosphatase type IV A member 3', located on chromosome 8q24.3),[140] TCEB1 (chromosome 8q21.11), and NOTCH signalling.[49] At present, the most convincing metastasis-regulatory gene in UM is BAP1, identified by exome sequencing.[139] The BAP1 gene encodes a deubiquitinating enzyme that binds to BRCA1 and BARD1 to form a tumour suppressor heterodimeric complex.[140] It is mapped to chromosome 3p21.31-p21.2, a region previously noted by Trolet et al[129] to be deleted in UM. BAP1 is implicated in other cancers,

Table 4 Most commonly used techniques for genetic typing of UM

Type of analysis	Molecular basis	Quantitative dosage analysis	Degree of automation	Cost	Remarks
Karyotype	Gross gain, loss, or alteration of chromosomes	+	+	+	Low-resolution copy number variation, cannot use FFPE
FISH	Gain or loss of small number of chromosome segments labelled with specific probes	+	+	+ +	Low-resolution copy number variation, can use FFPE
CGH	Gain or loss of large number of chromosome segments labelled with specific probes	+ +	+ +	+ + +	c-CGH: low-resolution a-CGH: high-resolution copy number variation both can use FFPE
MSA	LOH of a small number of highly polymorphic DNA segments	+ +	+ +	+	Low-resolution copy number variation/LOH, can use FFPE
SNP	LOH of moderately polymorphic DNA segments	+	+ + +	+ +	High-resolution copy number variation/LOH, can use FFPE
MLPA	Gain or loss of multiple chromosome segments	+ + +	+ + +	+ +	High-resolution copy number variation. 50 targets in one reaction can use FFPE
UM-GEP (PCR based)	Simultaneous measurement of mRNA expression of multiple genes	Not applicable	+ + +	+ + + +	Gene expression Can use FFPE, but fresh material preferred

Abbreviations: CGH, comparative genomic hybridisation; microsatellite analysis; multiplex ligation-dependent probe amplification; FFPE, formalin-fixed paraffin-embedded material; FISH, fluorescence *in situ* hybridisation; LOH, loss of heterozygosity; SNP, single-nucleotide polymorphisms; UM-GEP, uveal melanoma gene expression profiling.

which include mesothelioma, lung-, breast- and renal cell carcinomas.[141] Inactivating *BAP1* somatic mutations have been described in up to 84% of class 2 UM. Interestingly, germline *BAP1* mutations have been described in families with a high risk for hereditary cancer and a novel 'BAP1 cancer syndrome' including UM has been since been described by several groups.[142–146]

Besides *BAP1*, other genes are likely to be involved in the metastatic process, as indicated by our aSNP and whole exome sequencing of clinically and cytogenetically well-defined cohorts of UM with long-term follow-up (Lake *et al*, in press).[147]

Molecular alterations in UM metastases

Few cytogenetic or molecular genetic data are available on UM metastases. Trolet *et al*[129] examined 63 liver metastases using array CGH and showed these to be clustered in the same groups as the primary tumours but in differing proportions, with the 'class 2' gene signature as defined by this group. This is confirmed by studies examining UM metastases using FISH, demonstrating that the disseminated tumour cells are characterised by chromosome 3 loss and 8q gain.[109,113] We are currently examining paired primary and metastatic UM using aSNP and functional assays with the aim of identifying signalling pathways that are

aberrant within the metastases and, consequently, potential 'druggable targets'.

As outlined by Hanahan and Weinberg,[9,10] the metastatic process is multi-stepped and complex, and depends on numerous alterations occurring both within the tumour and its microenvironment. The latter is also of significance at the metastatic site, and often determines the location of metastases. This concept of 'seed and soil' has long been accredited to Stephen Paget;[148] it should actually be attributed to Ernst Fuchs, who interestingly described the notion of metastatic tropism 7 years earlier when discussing metastases of uveal tract 'sarcomas' (ie, melanomas).[149] This hepatic tropism of UM metastases remains unexplained but may be due to several factors including the chemokine receptor-ligand axis (eg, CXCR4 and CXCL12),[150] interactions between FAS and FAS-ligand,[151] IGF1 and IGF1-R, as well as C-Met and hepatocyte growth factor/scatter factor.[32,38]

Strategies for targeted therapy in UM metastases

It is beyond the scope of this article to summarise the various chemotherapeutic regimens applied in metastatic melanoma, which have been reviewed elsewhere.[8,152–154] It is, however, worth mentioning the potential targets for UM therapy, which have been revealed by efforts

unravelling key signalling cascades implicated in the development and progression of UM. These include the inhibitors of the: MAPK/MEK signalling pathway; PI3K/AKT pathway at the level of AKT; mTOR; mTOR blockade combined with an IGF-1R antibody; tyrosine kinases; c-Met pathway; CXCR4 and histone deacetylase.[154] The progress of these varied approaches will be followed with enthusiasm by clinicians, researchers, and patients alike.

Conclusions

Although considerable work has demonstrated that the 'hallmarks of cancer' are applicable to UM, the identification of the genetic pathways associated with UM oncogenesis and particularly those with metastasis is still at a preliminary stage. Characteristic copy number alterations and DNA expression profiles have been identified in UM, which are strongly correlated with prognosis. When these data are incorporated with the clinical and histomorphological features of UM in prediction models, a high degree of accuracy can be achieved for the individual patient, enhancing patient management.[134,155] It can be anticipated that the rapidly developing field of molecular genetics will shed further light on key signalling pathways involved in UM oncogenesis and progression, opening the way for target-based therapies.

Conflict of interest

The authors declare no conflict of interest.

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