Lipid-Producing Ciliochoroidal Melanoma with Expression of HMG-CoA Reductase

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Established Facts

- Lipid synthesis and metabolism can be key drivers of tumour survival.
- Lipid production has recently been described in uveal melanoma (UM).
- The mevalonate pathway is important in lipid metabolism, and HMG-CoA reductase is the rate-controlling enzyme; it is also the target of statins.

Novel Insights

- Lipid-producing UM may express HMG-CoA reductase, providing evidence for mevalonate pathway activity and cholesterol synthesis.
- Lipid-producing UM can have a high-risk molecular genetic signature.
- These tumour characteristics suggest novel targets, particularly inhibitors of the mevalonate pathway, as potential therapies for this unusual phenotype.

Keywords

Uveal melanoma · Lipids · Statin · Cholesterol · HMG-CoA reductase

Abstract

Uveal melanoma (UM) is the commonest primary intraocular malignancy in adults. There is limited published data on lipid production in UM. Here, we describe the clinical, histological, immunohistochemical, and molecular findings in a

ciliochoroidal melanoma with lipid production and expression of the enzyme HMG-CoA reductase. This case highlights an unusual UM tumour phenotype with a high-risk molecular metastatic profile and discusses tumour lipogenesis and activation of the mevalonate pathway as a potential therapeutic target in managing lipidised ciliochoroidal UM.

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Introduction

Uveal melanoma (UM) is the commonest intraocular malignancy in adults, with an incidence of 5.2 per million in the USA [1, 2] and up to 8.6 per million in Europe [3]. Up to 50% of the patients develop metastases. Survival for metastatic UM remains extremely poor with no effective treatments. Lipid synthesis and metabolism are known to play an important role in tumour survival in cancer cell biology [4]. Lipid production in UM has received relatively little research attention [5, 6]. In this report, we aim to add to this body of knowledge by describing the clinical, histological, immunohistochemical, molecular, and cytogenetic findings in a lipid-producing ciliochoroidal melanoma with a high metastatic risk. We examine tumour expression of HMG-CoA reductase and discuss mevalonate pathway inhibition as a possible therapeutic approach.

Case Report

A 39-year-old obese male patient with Type 2 diabetes mellitus and no background of cholesterol-lowering medication use presented with a 3-month history of blurry vision in the left eye. Full examination and B-scan ultrasonography revealed a superonasal ciliochoroidal melanoma with a base of approximately 16.0 mm and thickness 11.0 mm in the left eye with associated inferotemporal serous retinal detachment (Fig. 1a). The primary tumour was surgically managed by enucleation; however, it was not long before the patient developed metastases and was palliated. Macroscopically, the tumour had a minimally pigmented, yellow, and fatty cut surface with prominent vascular lakes (Fig. 1b, c). It measured 18 mm (base) × 12 mm (thickness) and extended from the pars plicata to within 3 mm of the optic disc.

On microscopy, the tumour had a nodular architecture (Fig. 1d) with vascular lakes and extravascular matrix loops forming networks. The tumour showed strong diffuse MART1 immunostaining (Fig. 1e) and loss of nuclear *BAP1* expression (Fig. 1f). Most tumour cells were epithelioid, and many had foamy cytoplasm consisting of intracytoplasmic vacuoles (Fig. 1g). The vacuoles did not contain glycogen (negative on PAS special staining; Fig. 1h) and were confirmed to consist of lipid on adipophilin immunohistochemistry (Fig. 1i). Many of the lipid-laden tumour cells also showed strong cytoplasmic HMG CoA reductase expression (Fig. 1j).

Immunohistochemistry was performed using routine diagnostic primary antibodies (online suppl. Table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000510393), proprietary consumables, and protocols based on the Ventana BenchMark Ultra platform (Roche Diagnostics, North Ryde, NSW, Australia).

Molecular cytogenetic CGH/SNP microarray was performed on fresh tumour material using the CytoScan 750K assay (Applied Biosystems, ThermoFisher, North Ryde, NSW, Australia) according to the manufacturer's protocol, and DNA was extracted using the Gentra Puregene kit (Qiagen). Analysis of the array results was performed using the Chromosome Analysis Suite (ChAS; version 3.1) and Mayrhofer et al. [7]. Monosomy for whole chromosome 3 was identified, together with whole arm gain of 8q (5 copies) and loss of 8p, consistent with 2 copies of an isochromosome 8q. Additional abnormalities included whole arm deletion of 16q, and sub-clonal segmental deletion 6q16.3q27 (~50% of the cells) and gain 16p11.2p13.3 (~20% of the cells; Fig. 1k).

To determine if there was a unique tumour mutational profile associated with this lipid-producing UM phenotype, DNA and RNA were extracted from formalin-fixed paraffin embedded tumour tissue and assayed by NGS using the OncoPrint Comprehensive and Tumour Mutation Load (TML) Assay on the Ion GeneStudio S5 platform (ThermoFisher; online suppl. Table S2). Coding region missense and nonsense variants with a variant allele frequency >0.05 with ≥20 variant reads and total coverage ≥400 reads were identified using Torrent Suite (version 5.10.1) and Ion Reporter (version 5.10.5.0) software (ThermoFisher). RNA data was assessed for the presence of potential gene fusions. Pathogenicity and clinical actionability were determined using Qiagen Clinical Insights version 1.4 (Qiagen, Chadstone, VIC, Australia) in accordance with ACMG and AMP/ASCO/CAP guidelines (Table 1) [8, 9].

RNA sequencing passed quality metrics, but no gene fusion events were detected. Missense mutations were detected in 7 known tumour-associated genes (Table 2; online suppl. Table S3). The presence of a *GNA11* mutation (c.626A>T p.[Gln209Leu]) confirmed the tumour's uveal melanocytic differentiation. Detection of a *BAP1* mutation (c.203A>G p.[Asp68Gly]) was consistent a high-risk UM mutational signature [10, 11].

Fig. 1. a B-scan ultrasonography showing a ciliochoroidal melanoma of the left eye with a base of approximately 15.93 mm and a thickness of 11.69 mm. **b, c** Macroscopic photography of the enucleated eye shows a ciliochoroidal tumour, a minimally pigmented, fatty cut surface, and conspicuous vascular lakes, measuring 18 mm (basal diameter) × 12.0 mm (height; **d**). H&E stained section showing a tumour with nodular architecture and epithelioid cytology, with **(e)** diffuse strong MART1 immunostaining and **(f)** loss of nuclear *BAP1* expression. **g** Higher power (×400) H&E section showing intracytoplasmic vacuoles, which were negative on PAS special staining **(h)** but stained positively for adipophilin on immunohistochemistry **(i)**. **j** HMG Co-A reductase staining is seen in the same vacuolated adipophilin+ tumour cells. **k** CGH/SNP analysis of the tumour revealed multiple molecular abnormalities, including monosomy for chromosome 3, gain of 8q (5 copies) with deletion of 8p. Subclonal deletion of 6q and of 16q were also detected.

(For figure see next page.)

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Table 1. Criteria to report clinically significant and pathological variants

No.	Criterion	Description
1	Sequencing quality metrics of the variant	<i>Is the variant truly present based on coverage and quality scores?</i>
2	Presence in the COSMIC database	<i>Has it been seen before in cancer?</i>
3	Absence from population databases	<i>Is the variant just a benign germline single-nucleotide polymorphism?</i>
4	Evidence of clinical actionability and/or pathogenicity	<i>Is the variant linked to a therapy or clinical trial, and is there evidence it causes loss or gain of gene function?</i>
Adapted from international standards and guidelines for the interpretation of sequence variants [8, 9].		

Table 2. Mutations identified in the primary case using the oncomine comprehensive and tumour mutation load assays

Gene	Mutation (HGVS)	Allele frequency	Coverage	Tier	Pathogenicity
<i>Oncomine comprehensive assay</i>					
<i>BAP1</i>	NM_004656.3(<i>BAP1</i>):c.203A>G p.(Asp68Gly)	52.21	>2,000	Tier 2C potential clinical significance	Likely pathogenic
<i>PIK3R1</i>	NM_181523.2(<i>PIK3R1</i>):c.1106C>T p.(Thr369Ile)	10.42	>2,000	Tier 3 unknown clinical significance	Variant of uncertain significance
<i>ATM</i>	NM_000051.3(<i>ATM</i>):c.1444A>C p.(Lys482Gln)	44.2	>2,000	Tier 3 unknown clinical significance	Variant of uncertain significance
<i>GNA11</i>	NM_002067.4(<i>GNA11</i>):c.626A>T p.(Gln209Leu)	26.64	>2,000	Tier 2C potential clinical significance	Pathogenic
<i>Oncomine TML assay</i>					
<i>BAP1</i>	NM_004656.3(<i>BAP1</i>):c.203A>G p.(Asp68Gly)	50.65	851	Tier 2C potential clinical significance	Likely pathogenic
<i>NSD2</i>	NM_001042424.2(<i>NSD2</i>):c.998C>T p.(Ala333Val)	44.74	1,292	Tier 3 unknown clinical significance	Variant of uncertain significance
<i>NFKB2</i>	NM_001077494.3(<i>NFKB2</i>):c.127G>A p.(Val43Met)	48.22	1,999	Tier 3 unknown clinical significance	Variant of uncertain significance
<i>ATM</i>	NM_000051.3(<i>ATM</i>):c.1444A>C p.(Lys482Gln)	49.9	525	Tier 3 unknown clinical significance	Variant of uncertain significance
<i>RARA</i>	NM_000964.3(<i>RARA</i>):c.838G>A p.(Glu280Lys)	47.59	353	Tier 3 unknown clinical significance	Variant of uncertain significance
<i>GNA11</i>	NM_002067.4(<i>GNA11</i>):c.626A>T p.(Gln209Leu)	28.31	1,148	Tier 2C potential clinical significance	Pathogenic

Tier refers to clinical actionability according to AMP/ASCO/CAP guidelines [9]. Pathogenicity was determined according to ACMG guidelines [8].

Discussion and Conclusion

It has been long recognized that chronic injury and degeneration due to ischaemia and inflammation can

contribute to lipidisation of neoplastic cells [4]. Lipogenic pathways were later shown to be upregulated in proliferating cells [12]. Importantly, altered lipid production has been shown to be a feature of cutaneous melanoma

[13], and high expression may be a prognostic factor associated with more advanced disease [14]. Recently, lipid production has also been demonstrated in a subset of UM, but the drivers of this lipidic profile remain unclear [5, 6].

The current report describes expression of HMG-CoA reductase in a lipid-producing UM with high-risk molecular cytogenetic (combined monosomy3 and high level 8q gain) [15, 16] and mutational signatures. HMG-CoA reductase is the rate-controlling enzyme of the mevalonate lipid metabolic pathway, which plays crucial roles in oncogenesis and cancer cell stemness [17, 18]. Enhanced cholesterol biosynthesis has been shown to play a role in prostate cancer development (reviewed by Hager et al. [19]), for example, and blocking the mevalonate pathway may have antineoplastic potential [20–22]. While epidemiological evidence on statin use and cancer survival are as yet inconclusive [23, 24], some preclinical trials of mevalonate pathway inhibitors have shown promising effects as primary or adjuvant therapy in several malignancies, including ovarian cancer [25, 26], breast cancer [22, 27], and renal cell carcinoma [28]. Despite these promising developments, clinical trials have not yet demonstrated consistently significant benefits for patients treated with mevalonate pathway inhibitors [29]. As a result, the potential of mevalonate pathway inhibitors as cancer therapy is yet unclear and remains an area of intense research interest. Our results raise the possibility that selecting patients for mevalonate inhibitor trials based on specific biomarkers of tumour phenotype (fatty macroscopic appearance, an abundance of adipophilin-expressing lipid vacuoles and HMG CoA reductase expression) may lead to more promising outcomes.

Our observations are limited to 1 case, and further studies are needed to fully interrogate lipid metabolic pathways in UM by multi-omic methods. In addition to the mevalonate pathway, futures studies would need to include glycerol phosphate, phospholipid, and eicosanoid lipid metabolic pathways. The patient in the current case was relatively young, obese, and diabetic, raising interesting questions about the effect of a lipogenic and obesogenic metabolic profile in the individual and its effect on UM development and biological behaviour. Future studies of UM are thus needed to examine sterol regulatory element-binding proteins, a family of transcription factors that are crucial for maintaining cellular lipid homeostasis (Bengoechea-Alonso and Ericsson, 2007). Aberrant activation of sterol regulatory element-binding proteins can contribute to obesity, fatty liver disease, and insulin resistance, and could also be involved in cancer development.

In conclusion, we demonstrate HMG-CoA reductase expression in a lipid-producing high-risk UM. Although lipid production likely confers a survival (and possibly metastatic) advantage, it does not appear to be associated with unique molecular cytogenetic or cancer-associated mutations. Our observations suggest statins deserve further investigation as adjuvant therapy in high-risk UM and that patient selection based on tumour biomarkers of cholesterol synthesis may impact the success of therapeutic trials.

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Statement of Ethics

Written informed consent was obtained from the patient for the use of his data for research and publication. This study is in compliance with the Declaration of Helsinki and was approved by the institutional committee on human research at St Vincent's Hospital (HREC Ref. 17-236).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

D.V.L., D.W., R.M.C., L.H., and S.C. conceived the study. D.V.L., D.W., R.M.C., L.H., R.L., and S.C. designed the experiments. D.V.L., D.W., M.G., R.M.C., S.L., R.L., L.H., and S.C. conducted experimentation and analysed the data. D.V.L., D.W., M.G., R.M.C., S.L., R.L., L.-A.L., L.H., and S.C. wrote and contributed to the manuscript.

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