PRDX3 is associated with metastasis and poor survival in uveal melanoma

Pathma Ramasamy ,[1,2,3] Anne-Marie Larkin,[2,4] Annett Linge,[2] Damien Tiernan,[5] Fionnuala McAree,[5] Noel Horgan,[5] Paul Moriarty,[5] Stephen Beatty,[6] Conor C Murphy,[1,5] Martin Clynes,[2,7] Susan Kennedy,[8] Paula Meleady[2]

[1]Department of Ophthalmology, Royal College of Surgeons in Ireland, Dublin, Ireland
[2]National Institute for Cellular Biotechnology, Dublin, Ireland
[3]Royal Victoria Eye and Ear Hospital, Dublin, Ireland
[4]Department of Life Sciences, Institute of Technology Sligo, Sligo, Ireland
[5]Royal Victoria Eye and Ear Hospital, Dublin, Ireland
[6]Waterford Institute of Technology, Waterford, Ireland
[7]Synthesis and Solid State Pharmaceutical Centre, Science Foundation Ireland, Dublin, Ireland
[8]Histopathology, Royal Victoria Eye and Ear Hospital, Dublin, Ireland

Correspondence to
Dr Pathma Ramasamy, Department of Ophthalmology, Royal College of Surgeons in Ireland, Dublin D02 YN77, Ireland; pathmaramasamy@rcsi.ie

This study was previously published as a thesis by Dr Pathma Ramasamy and is available online.[37]

Received 18 August 2019
Revised 25 October 2019
Accepted 8 November 2019
Published Online First 26 November 2019

To cite: Ramasamy P, Larkin A-M, Linge A, *et al*. *J Clin Pathol* 2020;**73**:408–412.

ABSTRACT

Aims Uveal melanoma (UM) is the most common primary intraocular malignancy in adults, and 40% develop fatal metastatic disease. Overexpression of thioredoxin-dependent peroxidase reductase (PRDX3) has been implicated in several cancers, including prostate, breast, colorectal and lung cancer. The aim of this study was to compare the immunohistochemical expression of PRDX3 in formalin-fixed, paraffin-embedded (FFPE) primary UM tissues of patients who did and did not develop metastatic disease.

Methods Immunohistochemical staining of PRDX3 was performed on FFPE tissue microarray samples of 92 primary UM tumours from patients who did and did not develop metastatic disease. The immunohistochemical staining was assessed by two observers who were blinded to all clinicopathological and cytogenetic details including metastatic/non-metastatic information. Based on a scoring system, expression of PRDX3 was graded as high or low.

Results There were 55 tumours (59.8%) from patients who developed metastatic disease, while 37 (40.2%) were from patients who did not develop metastasis. A statistically significant difference in PRDX3 expression was observed in patients who did and did not develop metastasis (p=0.001). A significant positive correlation between high PRDX3 expression and metastasis was observed (p=0.001). A significant negative correlation between PRDX3 expression and survival was found (p=0.005). Kaplan-Meier survival analysis showed a statistically significant difference in overall survival between tumours that demonstrated low and high expression of PRDX3 (67.61 vs 130.64 months, respectively, p=0.013).

Conclusions High immunohistochemical expression of PRDX3 in primary UM tissue is associated with metastasis and poor survival.

INTRODUCTION

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults. The overall incidence is approximately five to seven cases per million per year, and climbs to more than 20 cases per million per year by the age of 70.[1 2] The survival rates at 5, 10 and 15 years are 65%, 50% and 45%, respectively.[2–4] UM is associated with the development of metastasis in about 50% of cases, and 40% of patients with UM die of metastatic disease despite successful treatment of the primary tumour.[5 6] Metastatic spread occurs haematogenously, predominantly to the liver in up to 90% of patients with metastatic disease.[7] Other potential sites include lung, bone and skin, but these are rare in the absence of liver metastasis.[8] Despite progress in early diagnosis and treatment of primary UM, mortality rates have remained similar over the last 25 years.[9 10] This is due to the lack of effective biomarkers to identify early metastasis and therapeutic targets for metastatic UM.

The majority of studies to date attempting to understand the pathogenesis of UM have been performed at the genetic level. These have identified GNAQ and GNA11, which are mutations found in about 85%–91% of all UMs, representing the most common oncogenic mutation.[11–13] These mutations may represent an early event that leads to the development of UM. Primary UM clusters in two different genetic classes: class 1 tumours, which are associated with a good prognosis, and class 2 tumours with a high metastatic risk.[14 15] Further downstream, mutations in BAP1 gene located in chromosome 3 were found to occur almost exclusively in metastasising class 2 tumours.[16] Either BAP1 mutation or loss of chromosome 3 can occur first, but both events appear to be necessary for the tumour to metastasise.[17]

The peroxiredoxin (PRDX) family is critically involved in redox regulation of the cell and protects radical-sensitive enzymes from oxidative damage by a radical-generating system. In addition, these proteins are also involved in a range of other cellular roles, including the modulation of cytokine-induced hydrogen peroxide levels, which have been shown to mediate signalling cascades leading to gene expression, cell proliferation, differentiation and apoptosis.[18–20] Overexpression of thioredoxin-dependent peroxidase reductase (PRDX3) has been implicated in several cancers, including prostate, breast, hepatocellular, colorectal, lung and nasopharyngeal cancer, suggesting that it supports increased metabolism and protects proliferating cancer cells from apoptosis and chemodestruction.[21–28]

The objective of this study is to determine and compare the immunohistochemical expression of PRDX3 in formalin-fixed, paraffin-embedded primary UM tissues of patients who did and did not develop metastatic disease. Therefore, this is a retrospective study.

METHODS

Patient samples

Tissue specimens were obtained from the National Ophthalmic Laboratory, Royal Victoria Eye and Ear Hospital, Dublin, Ireland. Samples were also formalin-fixed and paraffin-embedded and cut in 4 µm sections for morphological assessment by immunohistochemistry. Cytogenetic analysis of chromosome 3 status was performed using fluorescent in situ hybridisation by the Merseyside and Cheshire Genetics Laboratory, Crown St, Liverpool, UK.

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue microarray (TMA) samples. Statistical power calculation showed that 202 patient samples were required for a power of 0.8. However, only a total of 92 primary UM tumours were available and were used. Each tumour had four representative cores. All immunohistochemical staining was performed using the Dako Autostainer Plus according to the manufacturer's instructions (Dako, Glostrup, Denmark). Dewaxing and antigen retrieval were done in the PT Link system (Dako) using Target Retrieval Solution pH 9. Endogenous peroxidase activity was blocked for 10 min with 200 µL of HRP Block (Dako). The slides were washed with 1X wash buffer and 200 µL of antibody solution added to the slides for 30 min for PRDX3 (GeneTex, Irvine, California (GTX111887); dilution 1:430 vol/vol). Antibody was diluted in Dako REAL Antibody Diluent. Slides were washed again with 1X wash buffer and then incubated with 200 µL REAL EnVision (Dako) for 30 min. Slides were washed again with 1X wash buffer and then stained with 200 µL 3-amino-9-ethylcarbazole substrate chromogen (Dako) for 5 min and this was repeated once more. All slides were counterstained with haematoxylin (Dako) for 5 min and rinsed with deionised water, followed by wash buffer. Once staining was completed, each slide was mounted with a coverslip using Faramount Mounting Solution (Dako). Negative control slides were incubated with Dako REAL Antibody Diluent only; the primary antibody was omitted.

The immunohistochemical staining was assessed by two observers who were blinded to all clinicopathological and cytogenetic details including metastatic/non-metastatic information (PR, AL). The TMA slides were scored based on staining only, as all cases demonstrated 100% staining given the small tumour core size. The staining intensities observed in tumours were assigned as negative, weak or strong. Each tumour had four representative cores, and each core was assigned a score of 0–2. No staining was scored as 0, weak staining as 1 and strong staining as 2. A total score for each patient was obtained by adding the scores of all four cores. Thus, a minimum score of 0 and a maximum score of 8 were obtained. The total score was divided into two categories: 0–3 as low expression and 4–8 as high expression. In order for a patient to be categorised as 'low expression', a minimum of at least one core per patient would be required to demonstrate negative staining. The minimum staining for a patient to be categorised as 'high expression' requires all four cores to demonstrate weak staining, one strong with two weak staining, or two strong with two negative staining tumour cores. Thus, tumours with heterogeneous PRDX3 staining in four cores would be classified as low or high based on the presence or absence of negative staining. Using this method, four weak staining cores would be appropriately categorised as a positive result. If a core was uninformative, missing, or contained no tumour tissue or exhibited extensive pigmentation, the overall score was that of the remaining core(s). Discordant scoring results were re-viewed by both observers together to reach agreement and a consensus score assigned.

All data were processed in SPSS V.22.0 for statistical analyses. Fisher's exact test (two-tailed), Pearson correlation and Spearman correlation were used to assess the association between clinical, histopathological and cytogenetic factors with immunohistochemical expression score. Differences of immunohistochemical expression score between samples of patients who developed and those who did not develop metastasis were examined by Mann-Whitney U test. Kaplan-Meier survival curves were produced for metastatic/non-metastatic information and PRDX3 expression based on immunohistochemical analysis in TMAs.

RESULTS

Immunohistochemical analysis of PRDX3 expression was performed on 92 primary UM TMA samples. The demographics, clinical, histopathological and cytogenetic details of 92 patient tumours analysed are outlined in table 1. Each UM tumour sample was represented by four tissue cores in the TMA slides. There were 55 tumours (59.8%) from patients who developed metastatic disease, while 37 (40.2%) were from patients who

Table 1 Demographics, clinical, histopathological and cytogenetic details of 92 uveal melanoma patient tumours analysed for immunohistochemical expression of PRDX3 using tissue microarray

	Metastasis	No metastasis	Total
Mean age of diagnosis (years)	61.81 (SD: 14.6), range: 31.25–93.75	56.76 (SD: 14.84), range: 24.75–85.0	59.77 (SD 14.82), range: 24.75–93.75
Dead	54/55 (98.2%)	14/37 (37.8%)*	68/92 (73.9%)
Mean survival (months)	64.04 (SD: 77.84), range: 3–427	189.07 (SD: 53.73), range: 96–328	89.78 (SD: 89.15), range: 3–427
Male	30 (61.2%)	19 (38.8%)	49 (53.3%)
Female	25 (58.1%)	18 (41.9%)	43 (46.7%)
Metastasis/no metastasis	55 (59.8%)	37 (40.2%)	92 (100%)
Liver	29 (52.7%)	–	–
Lung	3 (5.5%)	–	–
Brain	2 (3.6%)	–	–
Spine	2 (3.6%)	–	–
Multiple	6 (10.9%)	–	–
Site NA	13 (23.6%)	–	–
Ciliary body involvement	11 (78.7%)	3 (21.3%)	14 (15.2%)
Extrascleral extension			92
No	45 (57.7%)	33 (42.3%)	78 (84.8%)
Yes	6 (85.7%)	1 (14.3%)	7 (7.6%)
NA	4 (57.1%)	3 (42.9%)	7 (7.6%)
Cell types			92
Spindle	16 (51.6%)	15 (48.4%)	31 (33.7%)
Epithelioid	10 (76.9%)	3 (23.1%)	13 (14.1%)
Mixed	28 (59.6%)	19 (40.4%)	47 (51.1%)
NA	1 (100%)	0	1 (0.1%)
Tumour size			92
Small (<10 mm)	3 (33.3%)	9 (66.7%)	12 (13%)
Medium (10–15 mm)	13 (56.5%)	10 (43.5%)	23 (25%)
Large (>15 mm)	32 (69.6%)	14 (30.4%)	46 (50%)
NA	7 (63.6%)	4 (36.4%)	11 (12%)
Chromosome 3 status			17/92 (18.5%)
Monosomy 3	6 (60%)	4 (40%)	10 (58.8%)
Disomy 3	0	7 (100%)	7 (41.2%)
NA	49 (65.3%)	26 (34.7%)	75 (81.5%)

*Cause of death unrelated to uveal melanoma.
NA, not available; PRDX3, thioredoxin-dependent peroxidase reductase.

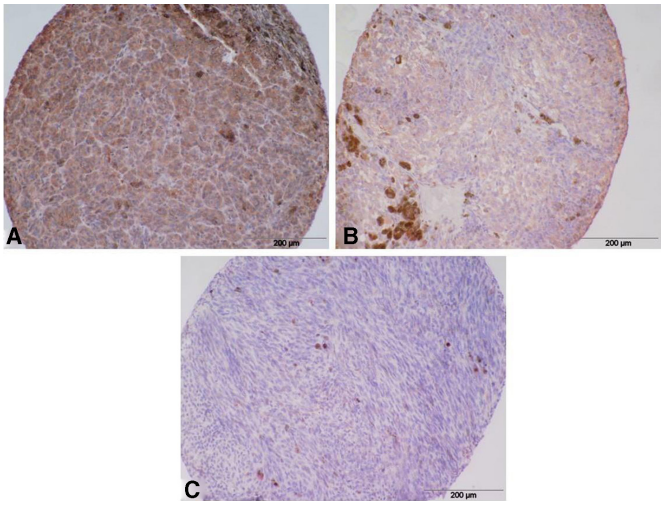

Figure 1 Low-power representative immunohistochemical images of PRDX3 expression in uveal melanoma tissue microarray samples showing (A) strong positive cytoplasmic PRDX3 staining, (B) weak positive cytoplasmic PRDX3 staining and (C) no PRDX immunoreactivity. Magnification ×200, scale bar=200 µm. PRDX3, thioredoxin-dependent peroxidase reductase.

did not develop metastasis. Patients who metastasised were diagnosed with UM between 1994 and 2010. The majority of patients without metastasis (31/37, 83.8%) were diagnosed with UM between 1994 and 2006 and were metastasis-free for a period of 7–19 years (up to 2013). There were two patients diagnosed in 2007 with monosomy 3 tumours who were metastasis-free for at least 6 years, and one patient diagnosed in 2009 with monosomy 3 tumour was metastasis-free for at least 4 years. A further three patients with disomy 3 tumours who were metastasis-free for at least 4 years were also included. Chromosome 3 information was available for 17 tumours; 10 were monosomy 3 and 7 were disomy 3 (58.8% and 41.2%, respectively). In patients with monosomy 3 tumours, 6 (60%) developed metastasis. The

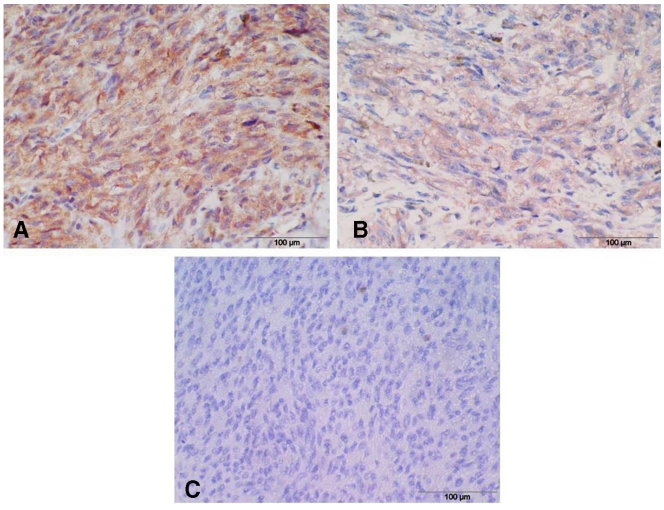

Figure 2 High-power representative immunohistochemical images of PRDX3 expression in uveal melanoma tissue microarray samples showing (A) strong positive cytoplasmic PRDX3 staining, (B) weak positive cytoplasmic PRDX3 staining and (C) no PRDX3 immunoreactivity. Magnification ×400, scale bar=100 µm. PRDX3, thioredoxin-dependent peroxidase reductase.

Table 2 Each tumour was represented by four tumour. The majority of tumours demonstrated the same intensity of staining in all four tumour cores. cores

	Number of cores staining with the same intensity		
	4	3	2
No metastasis	26/37 (70.3%)	10/37 (27%)	1/37 (2.7%)
Metastasis	47/55 (85.5%)	6/55 (10.9%)	2/55 (3.6%)

remaining four patients with monosomy 3 tumours, diagnosed between 2005 and 2009, were metastasis-free, while all seven patients with disomy 3 tumours did not develop metastasis. Figures 1 and 2 demonstrate representative strong positive, weak positive and negative staining in TMA tumour tissues.

The majority of tumours demonstrated the same intensity of staining across all four cores of tissue (26 of 37 (70.3%) tumours without metastasis and 47 of 55 (85.5%) tumours with metastasis). There were a number of tumours that demonstrated different staining intensities in the four cores of tissue. These are outlined in table 2. The mean score in the metastasised patients is 6.18 (SD: 2.66, 95% CI 5.46 to 6.90). The mean score in patient tumours with no metastasis is 4.54 (SD: 3.56, 95% CI 3.36 to 5.73).

There were 23 and 69 patient tumours with a score of 0–3 (categorised as low expression) and 4–8 (categorised as high expression), respectively. Seven and 48 patients who developed metastasis demonstrated low and high expression, respectively. Sixteen and 21 patients who did not develop metastasis demonstrated low and high expression, respectively (table 3). In the non-metastasised group, 4 of the 21 patient tumours that demonstrated high expression of PRDX3 are monosomy 3 tumours that were diagnosed between 2005 and 2009 (when chromosome 3 monosomy test was commenced as part of routine clinical practice). Although at the time of this study these patients were metastasis-free, it is highly likely that these patients will develop metastasis based on their cytogenetic risk factor of monosomy 3.

A statistically significant difference in PRDX3 expression was observed in patients who did and did not develop metastasis (p=0.001, Mann-Whitney U test; table 4). A significant positive correlation between high PRDX3 expression and metastasis was also observed (p=0.001, r=0.346, n=92, Spearman correlation). Furthermore, a significant negative correlation between PRDX3 staining score and expression with survival was found (p=0.005, r=−0.343, n=66; p=0.017, r=−0.294, n=66, respectively, Spearman correlation). No significant correlation was found between PRDX3 expression score and histopathological factors such as cell type and tumour size (0.797 and 0.313, respectively, Spearman correlation; table 5).

Survival analysis was performed to determine if PRDX expression was significantly associated with overall survival. In the 66 patients who have died, 14 demonstrated low expression while

Table 3 Distribution of PRDX3 expression score in primary uveal melanoma tumours with metastasis and without metastasis

PRDX3 expression	Metastasised tumours (n=55)	Non-metastasised tumours (n=37)	Total (n=92)
Low expression	7 (12.7%)	16 (43.2%)	23 (25%)
High expression	48 (87.3%)	21 (56.8%)	69 (75%)

Low expression is defined as a combined score of 0–3 and high expression is defined as a combined score of 4–8 for all four cores of tumour tissues per patient. PRDX3, thioredoxin-dependent peroxidase reductase.

Table 4 Test statistics showing statistically significant difference in PRDX3 expression in patients who did and did not develop metastasis

Test statistics	PRDX3 expression
Mann-Whitney U test	707
Wilcoxon W	1410
Z	−3.296
Asymptomatic significance (two-tailed)	0.001*

A total of 92 patients were studied, 55 with metastasis and 37 without metastasis.
*P<0.05.
PRDX3, thioredoxin-dependent peroxidase reductase.

52 demonstrated high expression (21.2% and 78.8%, respectively). The mean, SE and 95% CI for survival time for low and high expression of PRDX3 are outlined in table 6. A statistically significant difference in overall survival was observed between tumours that demonstrated low and high expression of PRDX3 (p=0.013, Mantel-Cox log-rank; p=0.026, Wilcoxon-Breslow; p=0.017, Tarone-Ware). Kaplan-Meier analysis demonstrated a significant negative correlation between PRDX3 expression and survival (figure 3).

DISCUSSION

Immunohistochemical analysis showed a significantly higher expression of PRDX3 in patients who developed metastasis. Cell type and tumour size have been associated with higher risk of metastasis. However, this study showed that the expression of PRDX3 was not associated with these histopathological factors. The difference in overall survival between tumours that demonstrated low and high expression of PRDX, demonstrated by Kaplan-Meier survival curve, was also significant.

Overexpression of PRDX3 has been implicated in several cancers, including prostate, breast, hepatocellular, colorectal, lung and nasopharyngeal cancer, suggesting that it supports increased metabolism and protects proliferating cancer cells from apoptosis and chemodestruction.[21–28] Chang *et al*[29] identified PRDX3, via its effects on H_2O_2, to be a critical regulator of apoptotic signalling. Depletion of PRDX3 resulted in increased intracellular H_2O_2, cytochrome c and other proapoptotic molecules such as caspase 3, sensitising cells to induction of apoptosis by staurosporine or tumour necrosis factor (TNF)-α.[29] Although many proapoptotic stimuli induce the intracellular accumulation of H_2O_2, a causal relationship between the mitochondrial generation of H_2O_2 and its active participation in apoptosis was shown.[29] Therefore, cells that express PRDX3, or indeed increased PRDX3 expression, may catalyse the production of TNF/staurosporine-mediated mitochondrial H_2O_2 necessary for apoptosis. Overexpression of PRDX3 protects thymoma cells from apoptosis induced by hypoxia, a bolus of peroxide or the anticancer agent imexon.[30] Another study identified PRDX3 overexpression in aggressive prostate cancer, where these cells demonstrated resistance to H_2O_2-induced apoptosis through a failure to activate proapoptotic pathways.[31 32] PRDX3, along with PRDX4, was also identified in a prostate cancer tissue proteomic study where these proteins were overexpressed and increased proliferation of prostate cancer cell lines.[33] An immunohistochemical study identified a positive correlation between PRDX3 expression and proliferation in breast cancer tissues. Silencing PRDX3 gene in breast cancer cell lines also decreased proliferation and induced cell cycle arrest.[34] Another study observed that the overexpression of PRDX I–III in breast cancer could be explained by the antiapoptotic and proliferative effects that these proteins exert.[35] Karihtala *et al*[36] found high expression of PRDX I, III, IV and V in breast carcinoma, suggesting that PRDXs are able to inhibit H_2O_2-mediated physiological apoptosis, cause abnormal proliferation and thereby may lead to tumourigenesis. Specifically, they found a correlation between strong PRDX3 expression and poorly differentiated tumours. The increased expression of PRDX3 in primary UM tissues from patients who developed metastasis found in this study indicates its potential role as a suppressor of mitochondria-mediated apoptosis by eliminating H_2O_2. Via this mechanism,

Table 5 PRDX3 expression is significantly associated with developing metastasis and poor survival and not significantly associated with cell type or tumour size

	PRDX3 expression, p value
Metastasis	0.001*, 0.001†
Survival	0.017†, 0.013‡
Cell type	0.797†
Tumour size	0.313†

*Mann-Whitney U test.
†Spearman correlation.
‡Mantel-Cox.
PRDX3, thioredoxin-dependent peroxidase reductase.

Table 6 Survival rates of patients with tumours that demonstrated low and high expression of PRDX3

	Survival (months)		
PRDX3 expression	Mean	SE	95% CI
Low (n=14)	130.64	24.77	82.14 to 179.14
High (n=52)	67.61	8.67	50.63 to 84.61
Overall (n=66)	80.99	9.09	63.17 to 98.80

A statistically significant difference in survival rate was observed between tumours that demonstrated low and high expression (p=0.013, Mantel-Cox log-rank; p=0.026, Wilcoxon-Breslow; p=0.017, Tarone-Ware).
PRDX3, thioredoxin-dependent peroxidase reductase.

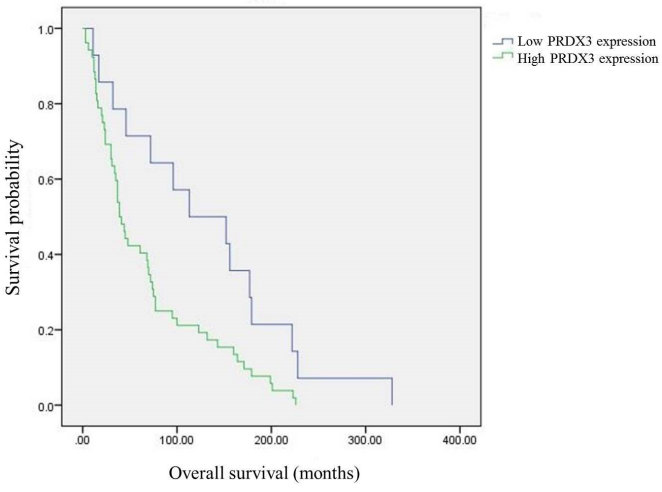

Figure 3 Kaplan-Meier survival analysis showing a significant negative correlation between PRDX3 expression and survival in 52 patients who demonstrated high expression compared with 14 patients who demonstrated low expression. The mean survival for patients with low and high PRDX3 expression is 130.64 and 67.61 months, respectively (p=0.013, Mantel-Cox log-rank; p=0.026, Wilcoxon-Breslow; p=0.017, Tarone-Ware). PRDX3, thioredoxin-dependent peroxidase reductase.

UM cells expressing high levels of PRDX3 may evade apoptosis, leading to uncontrolled cell proliferation. PRDX3 also protects the mitochondria against H_2O_2 and hydroxyl radical (OH^-)-mediated mitochondrial RNA damage. This supports the well-established knowledge that tumour cells exhibit a high metabolic rate to support rapid proliferation and growth.

To our knowledge, this is the first study that implicates PRDX3 in the metastatic phenotype of UM. Further analysis of the expression, activity level and function of PRDX3 in UM is essential for defining its potential role as a novel biomarker and identify therapeutic targets. Such studies could also provide new insights into the role of PRDX3 as a potential biological determinant contributing to the development of metastatic disease.

The retrospective nature and use of TMA samples limit the accuracy of this study. This study was underpowered, but this was unavoidable due to the limited number of tissue samples available and the rarity of UM. Furthermore, inclusion of only enucleated tumours (ie, larger tumours with worse prognosis) may also add bias to the study. Other limitations include those inherent to immunohistochemical studies, such as variable antibody reactivity, background staining and subjective interpretation. TMA rather than full UM tumour samples were used in order to preserve as much tissue as possible for further studies. However, the nature of this study was to determine expression of PRDX3 in UM tissues and enable future work to determine the significance of this protein in the molecular biology of this cancer.

Take home messages

- Uveal melanoma is the most common primary intraocular malignancy in adults, and 40% develop fatal metastatic disease.
- A significantly higher thioredoxin-dependent peroxidase reductase (PRDX3) expression was observed in patients who developed metastatic disease compared with those who did not.
- High PRDX3 expression was also associated with significantly shorter overall survival.

Handling editor Runjan Chetty.

Funding This research was funded by the Irish Health Research Board (grant no HRA-POR-2013-386) and the Royal Victoria Eye and Ear Research Foundation.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Ethical approval was obtained from the Research and Ethics Committee of the Royal Victoria Eye and Ear Hospital, Dublin. The research adhered to the tenets of the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository.

ORCID iD

Pathma Ramasamy http://orcid.org/0000-0001-9996-1394

REFERENCES

1 Egan KM, Seddon JM, Glynn RJ, *et al*. Epidemiologic aspects of uveal melanoma. *Surv Ophthalmol* 1988;32:239–51.
2 Singh AD, Topham A. Survival rates with uveal melanoma in the United States: 1973–1997. *Ophthalmology* 2003;110:962–5.
3 Jensen OA. Malignant melanomas of the human uvea: 25-year follow-up of cases in Denmark, 1943-1952. *Acta Ophthalmol* 1982;60:161–82.
4 Raivio I. Uveal melanoma in Finland. An epidemiological, clinical, histological and prognostic study. *Acta Ophthalmol Suppl* 1977;133:1–64.
5 Bedikian AY. Metastatic uveal melanoma therapy: current options. *Int Ophthalmol Clin* 2006;46:151–66.
6 Ehlers JP, Harbour JW. Molecular pathobiology of uveal melanoma. *Int Ophthalmol Clin* 2006;46:167–80.
7 Singh A, Borden E. Metastatic uveal melanoma. *Ophthalmol Clin North Am* 2005;18:143–50.
8 Lorigan JG, Wallace S, Mavligit GM. The prevalence and location of metastases from ocular melanoma: imaging study in 110 patients. *AJR Am J Roentgenol* 1991;157:1279–81.
9 Jemal A, Siegel R, Ward E, *et al*. Cancer statistics, 2006. *CA Cancer J Clin* 2006;56:106–30.
10 Singh A, Bergman L, Seregard S. Uveal melanoma: epidemiologic aspects. *Ophthalmol Clin North Am* 2005;18:75–84.
11 Van Raamsdonk CD, Griewank KG, Crosby MB, *et al*. Mutations in *GNA11* in Uveal Melanoma. *N Engl J Med* 2010;363:2191–9.
12 Onken MD, Worley LA, Long MD, *et al*. Oncogenic mutations in *GNAQ* occur early in uveal melanoma. *Invest Ophthalmol Vis Sci* 2008;49:5230–4.
13 Daniels AB, Lee J-E, MacConaill LE, *et al*. High throughput mass spectrometry-based mutation profiling of primary uveal melanoma. *Invest Ophthalmol Vis Sci* 2012;53:6991–6.
14 Onken MD, Worley LA, Ehlers JP, *et al*. Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death. *Cancer Res* 2004;64:7205–9.
15 Tschentscher F, Hüsing J, Hölter T, *et al*. Tumor classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities. *Cancer Res* 2003;63:2578–84.
16 Harbour JW, Onken MD, Roberson EDO, *et al*. Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science* 2010;330:1410–3.
17 Harbour JW. The genetics of uveal melanoma: an emerging framework for targeted therapy. *Pigment Cell Melanoma Res* 2012;25:171–81.
18 Hofmann B, Hecht H-J, Flohé L. Peroxiredoxins. *Biol Chem* 2002;383:347–64.
19 Butterfield LH, Merino A, Golub SH, *et al*. From cytoprotection to tumor suppression: the multifactorial role of peroxiredoxins. *Antioxid Redox Signal* 1999;1:385–402.
20 Fujii J, Ikeda Y. Advances in our understanding of peroxiredoxin, a multifunctional, mammalian redox protein. *Redox Report* 2002;7:123–30.
21 Wang Y-G, Li L, Liu C-H, *et al*. Peroxiredoxin 3 is resistant to oxidation-induced apoptosis of Hep-3b cells. *Clin Transl Oncol* 2014;16:561–6.
22 McDonald C, Muhlbauer J, Perlmutter G, *et al*. Peroxiredoxin proteins protect MCF-7 breast cancer cells from doxorubicin-induced toxicity. *Int J Oncol* 2014;45:219–26.
23 Shi L, Wu L-L, Yang J-R, *et al*. Serum peroxiredoxin3 is a useful biomarker for early diagnosis and assessment of prognosis of hepatocellular carcinoma in Chinese patients. *Asian Pac J Cancer Prev* 2014;15:2979–86.
24 Lin L-H, Xu Y-W, Huang L-S, *et al*. Serum proteomic-based analysis identifying autoantibodies against PRDX2 and PRDX3 as potential diagnostic biomarkers in nasopharyngeal carcinoma. *Clin Proteomics* 2017;14:6.
25 Liu Z, Hu Y, Liang H, *et al*. Silencing *PRDX3* Inhibits Growth and Promotes Invasion and Extracellular Matrix Degradation in Hepatocellular Carcinoma Cells. *J Proteome Res* 2016;15:1506–14.
26 Zhu J, Wu C, Li H, *et al*. Dach1 inhibits the proliferation and invasion of lung adenocarcinoma through the downregulation of peroxiredoxin 3. *Tumor Biol* 2016;37:9781–8.
27 Song I-S, Jeong YJ, Han J. Mitochondrial metabolism in cancer stem cells: a therapeutic target for colon cancer. *BMB Rep* 2015;48:539–40.
28 Song I-S, Jeong YJ, Jeong SH, *et al*. FOXM1-Induced Prx3 regulates stemness and survival of colon cancer cells via maintenance of mitochondrial function. *Gastroenterology* 2015;149:1006–16.
29 Chang T-S, Cho C-S, Park S, *et al*. Peroxiredoxin III, a mitochondrion-specific peroxidase, regulates apoptotic signaling by mitochondria. *J Biol Chem* 2004;279:41975–84.
30 Nonn L, Berggren M, Powis G. Increased expression of mitochondrial peroxiredoxin-3 (thioredoxin peroxidase-2) protects cancer cells against hypoxia and drug-induced hydrogen peroxide-dependent apoptosis. *Mol Cancer Res* 2003;1:682–9.
31 Whitaker HC, Patel D, Howat WJ, *et al*. Peroxiredoxin-3 is overexpressed in prostate cancer and promotes cancer cell survival by protecting cells from oxidative stress. *Br J Cancer* 2013;109:983–93.
32 Basu A, Banerjee H, Rojas H, *et al*. Differential expression of peroxiredoxins in prostate cancer: consistent upregulation of PRDX3 and PRDX4. *Prostate* 2011;71:755–65.
33 Ummanni R, Barreto F, Venz S, *et al*. Peroxiredoxins 3 and 4 are overexpressed in prostate cancer tissue and affect the proliferation of prostate cancer cells in vitro. *J Proteome Res* 2012;11:2452–66.
34 Chua P-J, Lee E-H, Yu Y, *et al*. Silencing the peroxiredoxin III gene inhibits cell proliferation in breast cancer. *Int J Oncol* 2010;36:359–64.
35 Noh DY, Ahn SJ, Lee RA, *et al*. Overexpression of peroxiredoxin in human breast cancer. *Anticancer Res* 2001;21:2085–90.
36 Karihtala P, Mäntyniemi A, Kang SW, *et al*. Peroxiredoxins in breast carcinoma. *Clin Cancer Res* 2003;9:3418–24.
37 Ramasamy P. Quantitative proteomic analysis and gene expression profiling microarray re-analysis of uveal melanoma. MD thesis. Available: https://epubs.rcsi.ie/mdtheses/63/