Laboratory science

Prognostic significance of pigmentation and stem cell markers in Indian population of uveal melanoma

Nikhil Kumar,[1] Mithalesh Kumar Singh ,[2] Lata Singh ,[3] Jayanti Jha,[1,1] Neelam Pushker ,[4] Neiwete Lomi,[4] Rachna Meel ,[4] Seema Sen ,[1] Seema Kashyap [1]

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[1]Ocular Pathology, All India Institute of Medical Sciences, New Delhi, India
[2]Dermatology, University of California, Irvine, California, USA
[3]Pediatrics, All India Institute of Medical Sciences, New Delhi, India
[4]Ophthalmology, All India Institute of Medical Sciences, New Delhi, India

Correspondence to
Professor Seema Kashyap; seemakashyap65@gmail.com and Dr Mithalesh Kumar Singh; mithalesh.aiims@gmail.com

MKS and SK contributed equally.
NK and LS contributed equally.

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ABSTRACT

Background Increased pigmentation has been associated with cancer stem-cell-like behaviour and chemoresistance in uveal melanoma (UM) and cutaneous melanoma. Therefore, our present study determines the correlation between pigmentation and cancer stem cell markers in UM patients.

Methods Expression of PAX3, CD133, ABCG2, TRYP1, TRYP2 and microphthalmia-associated transcription factor (MITF) was assessed by immunohistochemistry along with mRNA expression level of PAX3 using quantitative real-time PCR in 70 prospective UM cases. Kaplan-Meier analysis and Cox-proportional hazards model were used to analyse the correlation of protein expression with clinicopathological parameters and patient outcome.

Results We found significant expression of PAX3, CD133 and ABCG2 proteins in 28/70 (40%), 34/70 (49%) and 31/70 (44%) cases, respectively. There was a positive correlation between nuclear expression of PAX3 and high-risk clinicopathological parameters such as necrosis and scleral invasion ($p<0.01$). CD133 and ABCG2 expression were positively correlated with distant metastasis ($p=0.03$ and 0.01). Immunoexpression of PAX3, CD133 and ABCG2 proteins was positively correlated with MITF. TYRP2 expression correlated with PAX3 and ABCG2 immunoexpression. Tumour pigmentation was not correlated with any of the markers. PAX3 mRNA expression was positively correlated with immunoexpression of CD133 ($p<0.01$), ABCG2 ($p=0.01$) and distant metastasis ($p<0.01$). On Kaplan-Meier survival analysis, reduced metastasis-free survival was observed in patients with tumours showing high CD133 and ABCG2 expression. No significant correlation was observed between tumour pigmentation and overall survival.

Conclusion Our study highlights the association between PAX3 expression, pigmentation markers such as MITF and TRYP2 and cancer stem-cell markers in UM progression.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Increased pigmentation is a prognostic risk factor in uveal melanoma (UM). PAX3 regulates microphthalmia-associated transcription factor and influences UM progression. Stem cell markers are implicated in tumourigenesis and chemoresistance.

WHAT THIS STUDY ADDS

⇒ This study investigates the correlation between PAX3, CD133 and ABCG2 with the patient outcomes, offering insights into tumour heterogeneity and potential therapeutic targets.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings could advance UM research by identifying potential prognostic markers. In clinical practice, these markers may help in the development of new therapeutic strategies for UM.

INTRODUCTION

Uveal melanoma (UM) is the most common intraocular malignancy in adults, originating from the atypical melanocytes in the uveal tract. UM can be asymptomatic and is often diagnosed during routine ophthalmic examination.[1] Several clinical and histopathological parameters predict prognosis, including tumour size, epithelioid cell phenotype, lymphocytic infiltration, mitotic figures, etc. Frequent mutations in GNAQ/11 are the driver events for the development and progression of UM. BRCA-1 associated protein (BAP1) mutation is more prone to develop metastases in class 2 and monosomy 3 UMs. Preferentially expressed antigen in melanoma (PRAME) is a marker for increased risk of metastasis in class 1 and disomy 3 UMs. About 50% of patients develop liver metastasis via haematogenous spread, bypassing closer organs.[2]

Extensive research has focused on understanding the regulatory mechanisms of melanocyte development from neural crest-derived progenitor cells. Paired-box homeotic selector gene 3 (PAX3), a transcription factor, is one of the key players in melanoma tumourigenesis. It is expressed in melanocytes of normal skin coexpressed with known melanocyte markers. Expression of PAX3 has been observed in various human malignancies, including glioblastomas, neuroblastomas, rhabdomyosarcomas, Ewing's sarcoma, melanomas and nevi.[3–6] Its primary function is to regulate the survival and proliferation of melanoblast as well as regulate survival, differentiation and migration of melanocyte precursors.[7] PAX3 activates microphthalmia-associated transcription factor (MITF), a master gene that regulates

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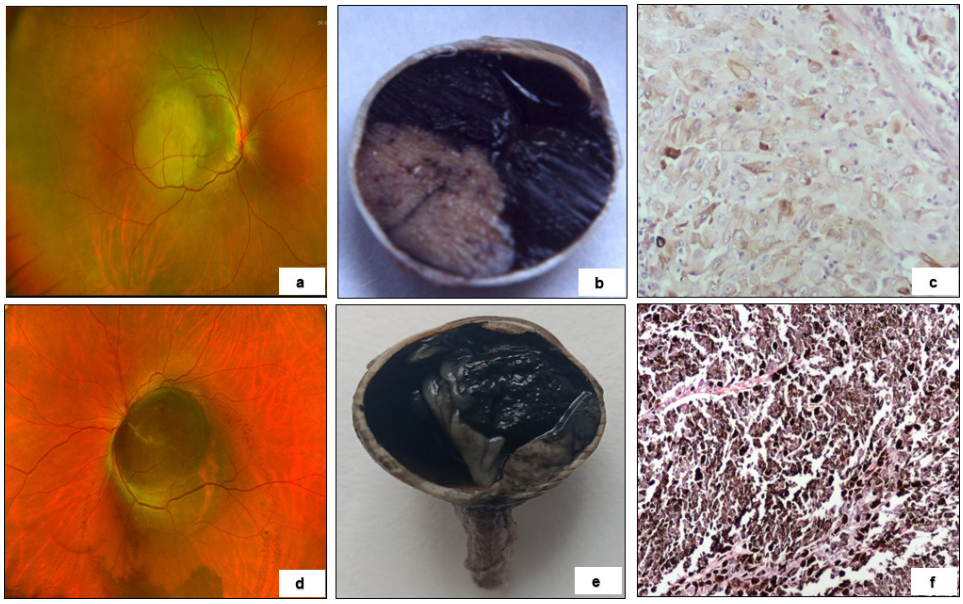

Figure 1 Representative images of clinical fundus examination, gross examination and H&E staining (×magnification): (a) clinical fundus picture of right eye showing low-pigmented choroidal melanoma; (b) gross picture of low-pigmented UM; (c) H&E image of low-pigmented UM (×200); (d) clinical fundus picture of left eye showing dome shaped highly pigmented choroidal melanoma; (e) gross picture of highly pigmented UM; (f) H&E image of highly pigmented UM (×200). UM, uveal melanoma.

of clinical fundus, gross examination and H&E staining, illustrating low and high-pigmented choroidal melanoma.

Immunoexpression of PAX3, CD133 and ABCG2 proteins

Expression of PAX3, CD133 and ABCG2 was observed in normal choroid and RPE (retinal pigment epithelium). The expression of PAX3 was weak in choroid and RPE as compared with CD133 and ABCG2. However, in UM cases, the expression of all the proteins (PAX3, CD133 and ABCG2) was strongly positive. Nuclear expression of PAX3 (nPAX3) was found in 40% (28/70) cases, and the cytoplasmic or membranous immunostaining of CD133 and ABCG2 was found positive in 49% (34/70) and 44% (31/70) cases, respectively (figure 2).

We have also compared the expression of proteins in high (n=50) and low (n=20) pigmented UM cases. High-intensity immunostaining of PAX3, CD133 and ABCG2 was found in high-pigmented cases as compared with low-pigmented cases (figure 3). High-pigmented cases showed expression of PAX3,

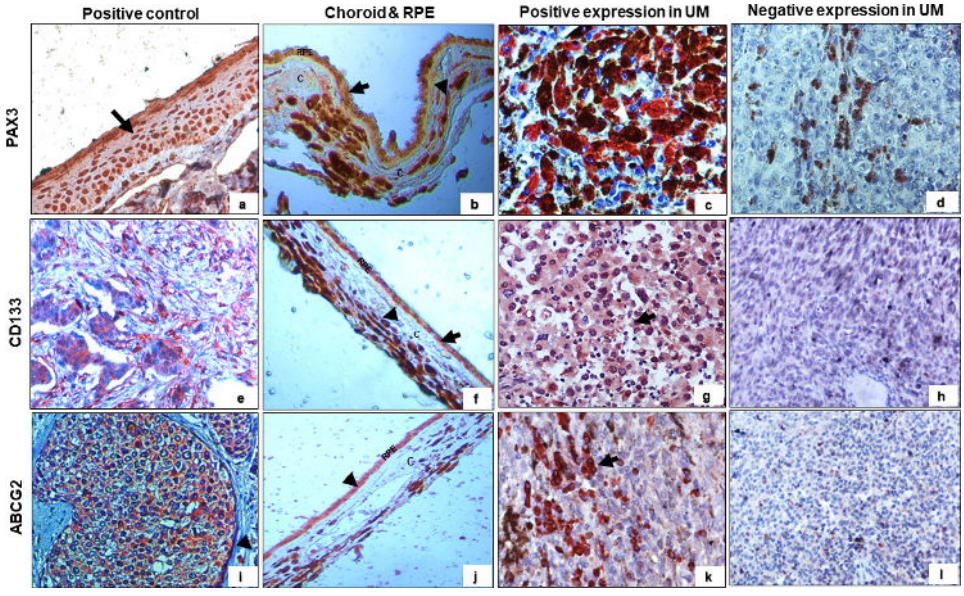

Figure 2 Immunohistochemical staining of PAX3, CD133 and ABCG2 in formalin-fixed tissue sections of the UM cases (×magnification): (a) PAX3 expression in normal skin served as antibody positive control (×200); (b) expression of PAX3 in RPE and choroid (×400); (c) positive expression of PAX3 in UM (×400); (d) negative expression of PAX3 (×400); (e) CD133 expression in breast carcinoma served as antibody positive control (×400); (f) expression of CD133 in RPE and choroid (×200); (g) positive expression of CD133 in UM (x200); (h) negative expression of CD133 (x200); (i) ABCG2 expression in breast carcinoma served as antibody positive control (x400); (j) expression of ABCG2 in RPE and choroid (x200); (k) positive expression of ABCG2 in UM (x200); (l) negative expression of ABCG2 (x200). RPE, retinal pigment epithelium; UM, uveal melanoma.

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