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CLINICAL STUDY

Demonstrating circulation in vasculogenic mimicry patterns of uveal melanoma by confocal indocyanine green angiography

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Abstract

Purpose **Vasculogenic mimicry patterns, formed by highly invasive melanoma cells, connect to endothelial cell-lined blood vessels and contain fluid *in vitro* and *in vivo*. This study was designed to determine if fluid leaks into vasculogenic mimicry patterns without circulation, or if fluid circulates in and clears from these patterns.**

Methods **Indocyanine green (ICG) laser scanning confocal angiography (Heidelberg Retinal Angiograph (HRA); Heidelberg Engineering, Heidelberg, Germany) was performed on nine patients with posterior choroidal melanoma in an institutional setting. Blood was drawn before the ICG injection and from the contralateral arm of the ICG injection site and 1 min after the injection. Outcome measures include time to first filling of retinal vessels and vasculogenic mimicry patterns and the time at which no fluorescence could be detected by the HRA instrument. After fluorescence was no longer detected in vessels or patterns, the tubes containing the patient's blood was imaged by the Heidelberg HRA.**

Results **Looping vasculogenic mimicry patterns were detected focally in five patients within 30 s after injection and were detectable up to 12 min post-injection. Blood drawn before ICG injection did not autofluoresce but ICG-containing blood pooled in the tube continued to fluoresce at 1-month post-injection.**

Conclusions **Vasculogenic mimicry patterns are not part of the endothelial cell-lined vascular system and fluid enters these patterns through leakage. The rapid infusion of ICG into these patterns after injection and the disappearance of fluorescence detectable by the Heidelberg HRA suggest that fluid circulates in these patterns and does not accumulate as a stagnant pool.**

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Keywords: uveal melanoma; vasculogenic mimicry patterns; confocal angiography; ICG; circulation

Introduction

The microcirculation of uveal melanoma is heterogeneous and complex. Lacking lymphatics,[1,2] uveal melanomas contain choroidal vessels that have been incorporated into the tumor, mosaic vessels (composed of vessels lined by tumor cells and endothelial cells), angiogenic vessels, and looping vasculogenic mimicry patterns.[2] Highly invasive and genetically deregulated melanoma cells generate patterns that loop around clusters of melanoma cells without participation by endothelial cells or fibroblasts.[3,4] These patterns are rich in laminin,[2,5] fibronectin,[6] collagens IV[7] and VI,[8] and a variety of glycosaminoglycans.[9,10] In human uveal melanoma tissue, vasculogenic mimicry patterns contain only trace amounts of collagen I and are clearly different from fibrovascular septa.[6]

Table 1 Imaging protocol

Time	*Action*
−1 min	Withdraw 3 ml of blood from left antecubital vein
0 min	Inject ICG into left antecubital vein
0–30 s	Angiographer identifies vasculogenic mimicry loops if present
30 s	Record images 10° or 20°, 1–2 frames per second for 3 min, and then at least every 30 s until no fluorescence detected in tumor microcirculation
1 min	Withdraw 3 ml of blood from right antecubital vein

Abbreviation: ICG, indocyanine green.

Non-endothelial cell-lined vasculogenic mimicry patterns have been shown to conduct fluid *in vitro*[3,4] and in animal models.[5,11] These patterns have also been visualized in patients with posterior choroidal melanomas by laser scanning confocal ophthalmoscopy with indocyanine green (ICG).[12,13]

The histological detection of looping vasculogenic mimicry patterns has been associated with an aggressive clinical course in multiple independent studies.[14–19] The presence of these patterns is associated with monosomy 3[20] (a cytogenetic marker of aggressive uveal melanoma behavior[20,21]) and a gene expression signature that is associated with the development of metastatic uveal melanoma.[22] The clinical detection of these patterns by confocal ICG angiography has been associated with the eventual growth of small and indeterminate posterior choroidal melanocytic lesions.[23]

Vasculogenic mimicry patterns, functioning as a 'fluid-conducting meshwork'[5] may also provide an alternative pathway to deliver therapeutic agents to uveal melanomas. Although fluid leaks from the endothelial cell-lined microcirculation into the non-endothelial cell-lined patterned extracellular matrix,[4,11] it is not known if fluid leaks and remains stagnant within vasculogenic mimicry patterns, or if fluid circulates through these patterns.[24] This study was designed to test the hypothesis that fluid circulates through vasculogenic mimicry patterns in posterior choroidal melanomas.

Methods

Ten consecutive patients with posterior choroidal melanoma, seen for the first time in the Ophthalmic Oncology Unit of the Hadassah-Hebrew University Medical Center between July 2005 and April 2006, were invited to undergo ICG angiography imaged with a Heidelberg Retinal Angiograph (HRA; Heidelberg Engineering, Heidelberg, Germany). One patient declined to participate because of sensitivity to iodine.

A volume of 3 ml of blood was drawn from the left antecubital vein into a tube without additives immediately before the intravenous injection of ICG, 25 mg/cm^3 of sterile aqueous solvent, at that site. For the first 30 s of the study, the angiographer attempted to identify vasculogenic mimicry patterns as defined by three back-to-back loops in each tumor. At 30 s, images were recorded at 10° or 20°, 1–2 frames per second for 3 min, and then at least every 30 s until no fluorescence was detected within the tumor microcirculation. At 1-min post-injection, 3 ml of the patient's blood was drawn from the right antecubital vein into a tube without additives and was stored in the dark at room temperature along with blood drawn before the injection of ICG. After the time point when no fluorescence was detectable with the HRA within the tumor, the patient's blood (pre- and post-injection) was imaged with the HRA at the same settings used for the confocal angiogram. The protocol is summarized in Table 1.

Informed consent was obtained. Digital images were stripped of all identifying (health protected) information and sent to the Department of Pathology at the University of Illinois at Chicago (RF and JL) for identification of vasculogenic mimicry patterns. The thickness of vasculogenic mimicry patterns was calculated from the 20° × 20° angiograms using methods outlined previously by Mueller *et al*.[13] Briefly, at a pixel resolution of 512 × 512 pixels, one pixel equals 11 × 11 μm for a 20° image.

This protocol was approved by the Helsinki Committee of the Hadassah-Hebrew University Medical Center and the Institutional Review Board of the University of Illinois at Chicago and was conducted in accord with HIPAA regulations and the tenets of the Declaration of Helsinki.

Results

Three back-to-back loops were detected in the tumors of five of nine patients studied. Within 12 min after injection, no fluorescence was detected within either the normal choroidal vessels within the tumor or within vasculogenic mimicry patterns. Figures 1, 2 and 3 illustrate a time-course sequence for one of these patients. No fluorescence was detected in blood drawn from patients before the injection of ICG. However, patient blood, drawn 1 min after injection, was

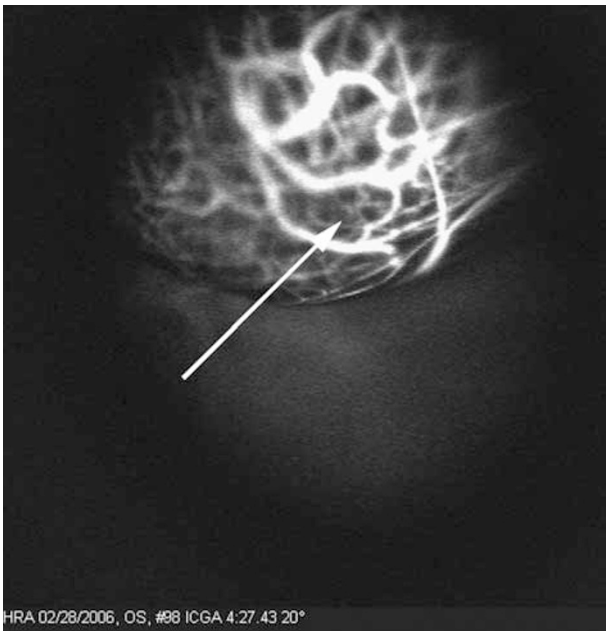

Figure 1 Vasculogenic mimicry loops are shown within a posterior choroidal melanoma (arrow) in this $20^\circ \times 20^\circ$ confocal ICG image taken at 4:27 min after ICG injection.

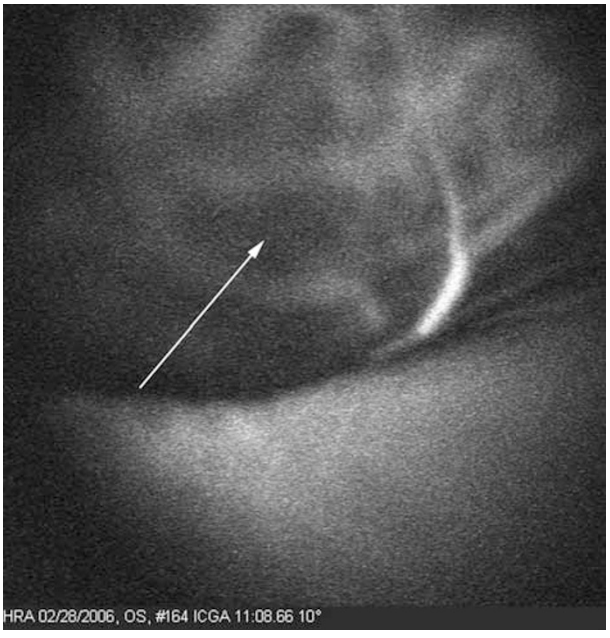

Figure 2 A $10^\circ \times 10^\circ$ confocal ICG image of the same tumor from Figure 1 taken at 11:08 min after the ICG injection. No fluorescence is visible within the vasculogenic mimicry loops (arrow).

fluorescent at 12 min post-injection, and continued to fluorescence for up to 4 weeks post-angiography. The thickness of the blood column in looping vasculogenic mimicry patterns was measured in this patient at 33 μm,

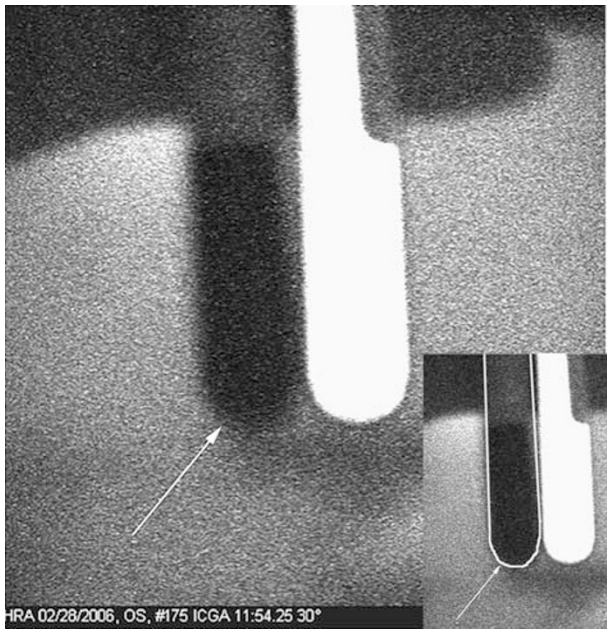

Figure 3 Blood was drawn from the patient 1 min before and after the ICG injection. A $30^\circ \times 30^\circ$ confocal ICG image taken was taken with the HRA at 11:54 min after the ICG injection under the same condition as the angiogram. Blood drawn before the ICG injection (arrow) does not fluoresce. Blood drawn after the injection, positioned to the right of the non-fluorescent tube, continued to fluoresce for 4 weeks after the injection. In the insert, the outline of the tube that contains blood that did not fluoresce is traced in white.

whereas the thickness of normal choroidal vessels in the same frame measured 253 μm. For the five patients whose tumors contained vasculogenic mimicry patterns, the mean thickness of the blood column in the patterns was 37.4 μm (SD 9.8) and the mean thickness of the blood column in the intralesional choroidal vessels was 233.2 μm (SD 47). In this limited data set, the differences between the thicknesses of vasculogenic mimicry loops and normal choroidal vessels approaches significance ($P = 0.0005$ for the paired t-test; $P = 0.0625$ for the sign test).

Discussion

Looping vasculogenic mimicry patterns have been shown to connect to endothelial cell-lined blood vessels in histological sections of human primary and metastatic uveal melanomas.[4,25] These patterns are not blood vessels. Three-dimensional reconstructions of vasculogenic mimicry patterns reveal them to be sheets of extracellular matrix proteins – especially rich in laminin – that wrap around branching cylinders of tumor cells.[25,26] The patterns are generated by melanoma cells and are not lined by endothelial cells.[3,4] Because it has

been shown repeatedly in animal models that intravenous tracers circulate not only through blood vessels but also through the non-endothelial cell-lined vasculogenic mimicry patterns, [5,9,11] it has been proposed that fluid leaks from tumor vessels into the patterns.[4,11] However, it is not known if the fluid that leaks into these patterns stagnates within them or circulates.

Mueller *et al*[13] demonstrated the presence of ICG within looping vasculogenic mimicry patterns in posterior choroidal melanomas by laser scanning ICG confocal angiography and correlated the angiographic pattern detection with the histological detection of looping periodic acid-Schiff (PAS)-positive patterns. Looping vasculogenic mimicry patterns are not normally detected in choroidal nevi. The blood column within these patterns tends to be considerably thinner than that seen in any choroidal vessel. Thus, it is unlikely that the patterns detected by ICG represent normal choroidal vasculature entrapped within uveal melanomas. In a prospective study, angiographic demonstration of these patterns in patients with posterior choroidal melanocytic lesions was associated with the subsequent growth of smaller indeterminate lesions.[23]

The angiographic detection of ICG within vasculogenic mimicry patterns does not address the issue of whether fluid leaks into the non-endothelial cell-lined extracellular matrix and remains stagnant, or if fluid circulates within the patterns. In this study, we observed that fluorescence within vasculogenic mimicry patterns dissipated within 12 min after intravenous injection of ICG, but blood drawn 1 min after the injection of ICG continued to fluoresce for up to 4 weeks post angiography. These data provide indirect evidence of the circulation of plasma through vasculogenic mimicry patterns in human posterior choroidal melanomas. The presence of an active circulation through vasculogenic mimicry patterns is significant because it has been shown recently that these patterns provide for at least an 11-fold increase in surface area over tumor blood vessels.[26] Therefore, vasculogenic mimicry patterns may provide for a more effective delivery route of therapeutic agents than endothelial cell-lined blood vessels.

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