

5-S-Cysteinyl-dopa as Diagnostic Tumor Marker for Uveal Malignant Melanoma

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Purpose: To evaluate the clinical significance of 5-S-cysteinyl-dopa (5-S-CD), a major intermediate in melanin synthesis, as a potential diagnostic tumor marker for uveal malignant melanoma.

Methods: The levels of 5-S-CD in the serum were measured by high-performance liquid chromatography in 16 patients with primary uveal melanoma. The levels of 5-S-CD were also measured in both aqueous and vitreous humor in 10 patients with uveal melanoma. The serum of healthy volunteers and patients with skin diseases other than melanoma, and the intraocular fluids of patients with cataract and vitreoretinal diseases were used as controls.

Results: Serum concentrations of 5-S-CD in patients with uveal melanoma in the absence of extraocular metastases were close to those of controls; however, serum concentrations of 5-S-CD were significantly elevated in patients with extraocular metastases of melanoma. Concentrations of 5-S-CD in the intraocular fluids, especially vitreous humor, were higher in patients with uveal melanoma than in controls.

Conclusions: 5-S-CD in intraocular fluids may serve as a useful biochemical marker for the diagnosis of uveal melanoma. Serum 5-S-CD may contribute to the assessment of the presence and progression of extraocular metastases in patients with uveal melanoma. **Jpn J Ophthalmol 2001;45:538–542** © 2001 Japanese Ophthalmological Society

Key Words: 5-S-CD, diagnosis, tumor marker, uveal melanoma.

Introduction

Uveal melanoma is a relatively common intraocular malignant neoplasm in adults, with an incidence of 6 to 7 cases per million people in the United States^{1–3} and 0.25 cases per million in Japan.⁴ In general, diagnosis of primary uveal melanoma is made by its characteristic ocular manifestations obtained by slit-lamp biomicroscopy, indirect ophthalmoscopy, fluorescein angiography, and other diagnostic methods. Radiological examination, including magnetic resonance imaging, may also play an important role in the diagnosis of uveal melanoma.⁵ However, there are some cases that are difficult to diagnose

due to atypical ocular manifestations or accompanying intraocular changes, such as extensive retinal detachment, vitreous hemorrhage, and other complications.⁶ Histopathological examination without enucleating the eyeball is one ideal method to obtain the accurate diagnosis of uveal melanoma. While fine needle biopsy or excisional biopsy according to certain protocols can yield a histopathologic diagnosis,^{7,8} these diagnostic or therapeutic techniques are applicable only in a limited number of cases. In addition, the follow-up of patients with uveal melanoma after diagnosis and treatment, including detection of local recurrence and extraocular metastases is controversial.^{9,10}

We have already reported the significance of 5-S-cysteinyl-dopa (5-S-CD),¹¹ which is known as a major intermediate in pheomelanin synthesis,^{12,13} in the diagnosis of uveal melanoma. In the present study, 5-S-CD was measured in peripheral blood as well as

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in intraocular fluids of patients with primary uveal melanoma, as an extension of the research in our previous report¹¹ to determine whether 5-S-CD could be a potential biochemical tumor marker.

Materials and Methods

We reviewed the records of 16 patients with primary uveal melanoma diagnosed at the Department of Ophthalmology of Tokyo Medical University between 1992 and 1999 (Table 1). Serum specimens had been prepared by centrifugation of peripheral blood drawn in the absence of heparin. Four of the 16 cases showed extraocular metastases at the time of serum preparation and sequential analysis was performed in 3 of the 4 cases. Controls consisted of serum samples from healthy volunteers (aged 18–44 years) and from patients with skin diseases other than melanoma (aged 53–84 years), which had been obtained in a previous study.¹⁴

Aqueous and vitreous humor were obtained from 10 patients with uveal melanoma immediately following eyeball enucleation. Aqueous humor (150–200 mL) was aspirated from the limbus using a 27-gauge needle, and 500–1,000 mL of vitreous humor was drawn using a pipette after dissection of the

sclera, taking care to avoid cutting the tumor. Control aqueous humor was obtained from 24 patients with senile cataract at the time of cataract surgery. Vitreous humor samples obtained at vitrectomy from patients with vitreoretinal diseases, including macular hole, epiretinal membrane, diabetic retinopathy, and uveitis were also used as controls. Both serum and intraocular fluids were stored at –80°C until use.

The 5-S-CD in the serum and intraocular fluids was extracted by alumina treatment¹⁵ and measured by the high-performance liquid chromatography (HPLC) method (Figure 1). Briefly, we used the Pu-980 intelligent pump, an 851-AS intelligent sampler, an 840-EC electrochemical detector with a potential of +750 mV versus a hg/Hg₂Cl₂ reference electrode and a 4.6-mm (inside diameter) × 150-mm Catecholpak C¹⁸ reserved-phase column, 7-μm particle size (all from Jasco, Tokyo) for the HPLC analysis. The mobile phase contained 12 g of 85% phosphoric acid, 10 g of methanesulfonic acid, and 0.1 mmol of Na₂EDTA per liter of water, the pH being adjusted to 3.10 with 5 mol/L NaOH. The flow rate was 0.7 mL/min. Stock solutions of 5-S-CD (5 μmol/L) and of the internal standard Me-CD (15 μmol/L) were prepared in 0.1 mol/L HCl containing 1 g/L each Na₂EDTA, and stored at –30°C until use.¹⁶

Table 1. Values of 5-S-Cysteinyldopa (5-S-CD) in Serum and Intraocular Fluids in Patients with Uveal Melanoma

Case No.*/ Age (yrs)/ Sex	Site	Tumor Size (mm)	Treatment	Histopathology	5-S-CD (nmol/L) [†]			Extraocular Metastases	Follow-up Period (months)
					Serum	Aqueous Humor	Vitreous Humor		
1/72/F	Iris	2 × 2	Observation	Unknown	21.3	ND	ND	Unknown	23
2/75/M	Iris/ciliary body	4 × 3	Observation	Unknown	4.5	ND	ND	None	48
3/69/F	Choroid	14 × 8	Enucleation	Spindle B type	4.5	ND	ND	None	94
4/76/M	Choroid	13 × 9	Enucleation	Mixed type	206	ND	ND	Liver	62 [‡]
5/50/F	Ciliary body/choroid	15 × 8	Local resection	Epithelioid type	13	ND	ND	Liver, brain, pelvis	50 [‡]
6/46/M	Choroid	7 × 4	Enucleation	Mixed type	81.6	ND	ND	Liver	71 [‡]
7/74/F	Choroid	6 × 6	Enucleation	Mixed type	4.1	ND	ND	None	57
8/62/M	Choroid	12 × 6	Enucleation	Mixed type	4.8	13.1	55.8	None	44
9/63/M	Choroid	7 × 3	Photocoagulation	Mixed type	4.5	7.9	8.2	None	61
10/67/F	Choroid	16 × 12	Enucleation	Mixed type	5.9	1221	1840	None	48
11/65/M	Choroid	14 × 14	Enucleation	Mixed type	4.4	ND	ND	None	43
12/82/M	Choroid	16 × 15	Enucleation	Epithelioid type	31.9	9.7	34.3	Liver	6 [‡]
13/52/M	Choroid	13 × 10	Enucleation	Spindle B type	4.8	5.3	25.6	None	23
14/47/F	Choroid	12 × 10	Enucleation	Spindle B type	3	5.2	14.8	None	20
15/37/M	Choroid	13 × 11	Enucleation	Spindle B type	3	5.1	8.9	None	20
16/77/F	Ciliary body/choroid	Diffuse	Enucleation	Mixed type	4	17.3	56.7	None	30

*Cases 1 to 10 were reported previously.¹¹ Case 1 died from renal failure. 5-S-CD in serum of case 5 was measured after surgical removal and radiation therapy for extraocular metastases. 5-S-CD in intraocular fluids of case 9 was measured after photocoagulation therapy.

[†]ND: not determined.

[‡]Period until metastases.

Results

The mean (\pm SD) concentrations of 5-S-CD in the controls were 4.3 ± 1.8 nmol/L in serum,¹⁴ 8.7 ± 2.6 nmol/L (ranging from 4.9 to 15.5 6 nmol/L) in aqueous humor, and 4.7 ± 2.1 nmol/L (ranging from 1.3 to 9.5 6 nmol/L) in vitreous humor (Table 2).

Serum concentration of 5-S-CD in patients with uveal melanoma without extraocular metastases ($n = 12$) was 4.3 ± 0.8 nmol/L, close to those of the con-

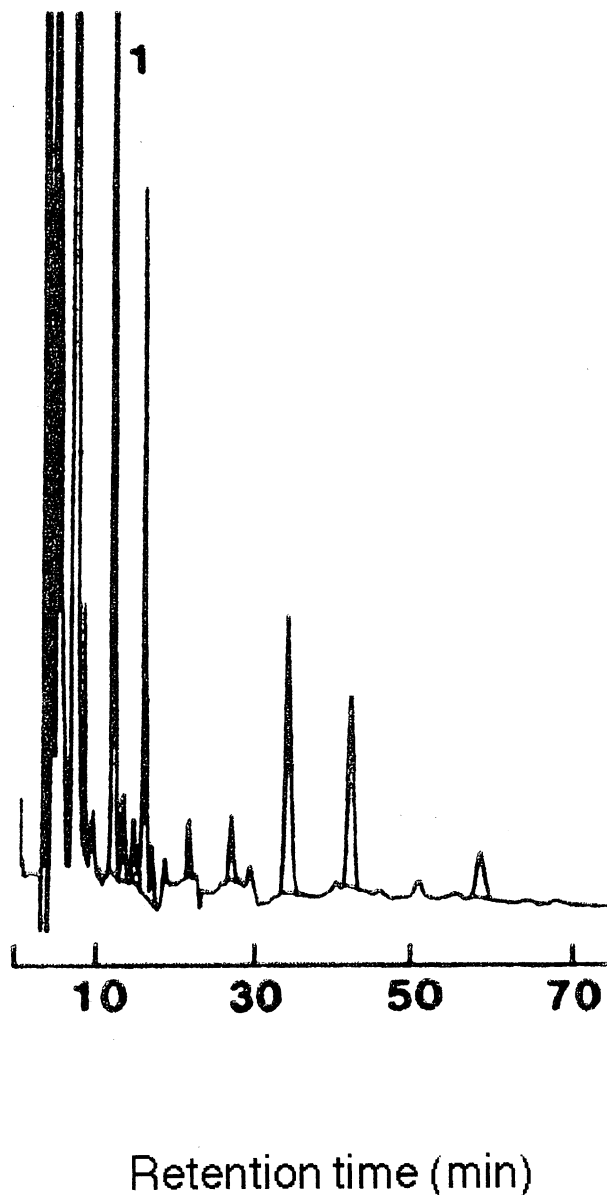


Figure 1. Typical high-performance liquid chromatogram of pooled serum sample from patients with metastatic skin melanoma. 1: 5-S-cysteinyl dopa.

Table 2. Values of 5-S-Cysteinyl dopa (5-S-CD) in Serum and Intraocular Fluids of Controls

Samples	5-S-CD (nmol/L)
Serum ($n = 33$)	4.3 ± 1.8
Aqueous humor ($n = 24$)	8.7 ± 2.6
Vitreous humor ($n = 23$)	4.7 ± 2.1

trois; whereas serum concentrations of 5-S-CD were found to be elevated in patients with extraocular metastases of uveal melanoma ranging from 13.0 to 206 nmol/L (Table 1). Patients with extraocular metastases, sequentially followed by measurement of serum 5-S-CD, revealed gradual increase of the value in parallel to the progression of extraocular metastases. In 2 cases, the elevation of serum 5-S-CD was recognized prior to the detection of extraocular metastases by periodical systemic examinations, including abdominal echography and computed tomography (Figures 2 and 3).

Concentrations of 5-S-CD in intraocular fluids, especially vitreous humor, showed a wide distribution range and were elevated more than 2 SD in 6 of 8 patients with uveal melanoma, whereas concentrations of 5-S-CD in the aqueous humor varied from normal levels to high values.

Discussion

Tumor markers are clinically utilized to detect occult metastases and recurrence, evaluate prognosis, and monitor response to therapy. The traditional approach to tumor markers that have been studied is based on the detection of substances that are either induced by or released by cancer cells.¹⁷ New diag-

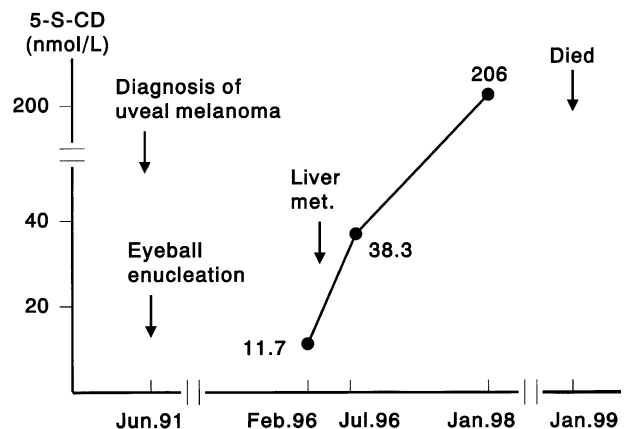


Figure 2. Clinical course and serum 5-S-cysteinyl dopa (5-S-CD) in case 4. Liver metastasis was revealed by abdominal computed tomography scan.

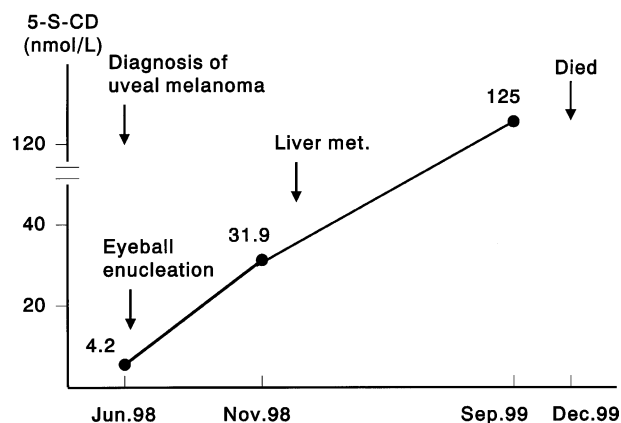


Figure 3. Clinical course and serum 5-S-cysteinyl dopa (5-S-CD) in case 12. Liver metastasis was revealed by abdominal echography and computed tomography scan.

nostic techniques in molecular biology have been applied in various cancers and have contributed to the early detection of metastases. Tyrosinase mRNA is one of the representative new molecular markers for the diagnosis of malignant melanoma¹⁷⁻¹⁹; however, most of these new tumor markers may be of limited value in the diagnosis and management of patients, and have not yielded clinically useful levels of accuracy.¹⁹

It is known that 5-S-CD is one of the metabolites in the pathway of the synthesis of pheomelanin in normal melanocytes as well as in melanoma cells.²⁰ Several clinical studies have suggested that serum 5-S-CD could be the most useful biochemical marker among the major melanocytic metabolites, including 5,6-dihydroxyindole-2-carboxylic acid (5,6DHI2C), for the detection or progression of systemic melanoma.^{12,21,22} Most of the studies using 5-S-CD as a tumor marker performed in the past have concentrated on melanoma arising from the skin,^{21,22} and the significance of 5-S-CD in patients with uveal melanoma has remained unclear.

Previous reports suggested that a 5-S-CD value of more than 10 nmol/L in the serum is an alarming sign of metastases of skin melanoma.¹⁴ In this study, extraocular metastases were demonstrated in 4 of 16 patients, and in all 4 cases the 5-S-CD values exceeded 10 nmol/L in the serum. In contrast, no extraocular metastases have been detected in patients with a 5-S-CD value of less than 10 nmol/L in the serum. The fact that elevation of 5-S-CD in serum was recognized prior to the detection of extraocular metastases by systemic examinations, including radiologic examinations, indicated the potential diagnostic efficacy of serum 5-S-CD to detect the early stage of extraocular metastases in the patients with uveal

melanoma (cases 4 and 12). However, values of 5-S-CD in the serum of patients with uveal melanoma without extraocular metastases were close to those of controls, indicating that measurement of serum 5-S-CD may not be useful for the early diagnosis of primary uveal melanoma.

In intraocular fluids, especially vitreous humor, 5-S-CD was elevated in most patients with uveal melanoma, regardless of the presence or absence of extraocular metastases. Only 2 cases (cases 10 and 15) revealed values of 5-S-CD close to those of controls. Case 9 (5-S-CD value of 8.2 nmol/L) had a history of treatment by photocoagulation, resulting in refractory vitreous hemorrhage before eyeball enucleation and sampling of the intraocular fluids. Histopathological examination of the enucleated eyeball in this case revealed necrotic melanoma cells and proliferation of collagen tissue at the site of tumor, and these histological changes were compatible with the low level of 5-S-CD in intraocular fluids.

The value of measuring 5-S-CD seems to be less in aqueous humor than in vitreous humor. The higher value of 5-S-CD in vitreous humor than in aqueous humor may reflect the direct secretion of 5-S-CD from the choroidal melanoma into the vitreous cavity or an alteration of dynamics and turnover of the intraocular fluids in the eye. Variation of the 5-S-CD value in intraocular fluids in individual cases may be affected by the activity of melanin synthesis, pigment dispersion from the tumor into the eye, or other factors.

In this study, 2 cases of iris nevus with senile cataract whose aqueous humor samples were used as controls had normal values of 5-S-CD. However, the significance of 5-S-CD in intraocular fluids in patients with other melanocytic tumors, such as melanocytoma and choroidal nevus, is obscure at present. The value of 5-S-CD in the intraocular fluids in patients with amelanotic uveal melanoma is also unknown. While further investigation regarding the specificity and sensitivity of 5-S-CD as a diagnostic tumor marker for uveal melanoma is required, nevertheless, 5-S-CD measurement appears to hold significant future promise in the definitive diagnosis of this disease.

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