

Cytokines concentrations in aqueous humor of eyes with uveal melanoma

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Abstract

Uveal melanoma (UM) is the major intraocular malignancy in adults, of which the molecular biology is still unknown. Therefore, this study was designed to determine the aqueous concentrations of angiogenic, inflammatory, and chemotactic cytokines in eyes with UM.

Aqueous humor samples were collected from 38 patients with UM and 22 patients undergoing cataract surgery. Interleukin 6, 8 (IL-6, IL-8, respectively), interferon-inducible protein-10 (IP-10), placental growth factor1 (PIGF1), regulated on activation, normal T Cell expressed and secreted (RANTES), monocyte chemoattractant protein-1 (MCP-1), nerve growth factor-beta (NGF- β), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and vascular endothelia growth factor A (VEGF-A) were assessed by multiplex bead assay.

In the study group, significantly higher concentrations of IL-6 ($P = .006$), IL-8 ($P = .018$), IP-10 ($P = .004$), RANTES ($P = .008$), MCP-1 ($P = .02$), NGF- β ($P = .013$), EGF ($P < .001$), PIGF1 ($P = .01$), bFGF ($P = .016$), and VEGF ($P = .017$) were measured, when compared with the control group.

Several angiogenic, inflammatory, and chemotactic cytokines are highly expressed in the aqueous humor of the UM eyes, which provides new insights into the pathophysiology of UM and could be potential targets for treatment.

Abbreviations: bFGF = basic fibroblast growth factor, EGF = epidermal growth factor, EGFR = epidermal growth factor receptor, IL = interleukin, IP-10 = interferon-inducible protein-10, MCP-1 = monocyte chemoattractant protein-1, NGF- β = nerve growth factor-beta, PIGF1 = placental growth factor1, RANTES = regulated on activation, normal T Cell expressed and secreted, sVCAM = soluble vascular cell adhesion molecule, TAMs = tumor-associated macrophages, UM = uveal melanoma, VEGF-A = vascular endothelia growth factor A.

Keywords: aqueous humor, cytokines, uveal melanoma

1. Introduction

Uveal melanoma (UM) is the most common intraocular malignancy in adults with a poor prognosis because of a high incidence of metastases, of which the 5-year survival rate is about 60%.^[1-4] Although many therapies have been developed to keep excellent local control, little progress has been made to alter the disease course^[5] and improve the survival rate.^[6,7] In metastatic

UM, systemic chemotherapy is usually unsuccessful. To improve the prognosis of UM, the molecular mechanisms involved in UM progression and metastasis should be cleared.

Recently, a few studies indicated that vascular endothelia growth factor A (VEGF-A) and basic fibroblast growth factor (bFGF) were highly expressed in human eyes with UM.^[8-10] As the metastasis of UM is mainly hematogenous, angiogenesis probably plays a crucial role in UM. Additionally, mounting evidence indicates that inflammatory processes may involve in the UM progression. Macrophages were detected variable in UM by several studies. A high density of tumor-associated macrophages (TAMs) probably indicates poor prognosis.^[11] Meanwhile, UM cells could produce inflammatory cytokines such as IL-6 and IL-10, which induce TAMs differentiation to the M2-type. M2-type was considered to have a tumor-promoting role rather than an effective immune response. The infiltrating macrophages could also produce VEGF, which indicated macrophages probably play roles in angiogenesis and Inflammation. Therefore, in this study, we determined the aqueous concentrations of angiogenic, inflammatory, and chemotactic cytokines in UM patients, and estimated their potential implications in the pathogenesis of UM.

2. Methods

The Ethics Committee and Institutional Review Board of Peking University People's Hospital approved this study, and written informed consent was obtained from each patient in accordance with the Declaration of Helsinki. All the patients were diagnosed at Peking University People's Hospital. Exclusion criteria included previous intraocular surgery, concomitant ocular diseases, concomitant systemic diseases such as hypertension

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The Ethics Committee and Institutional Review Board of Peking University People's Hospital approved this study, and written informed consent was obtained from each patient in accordance with the Declaration of Helsinki.

The authors have no conflicts of interest to disclose.

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or diabetes, prior cardio-cerebrovascular accidents within the past 6 months. Undiluted aqueous humor samples were collected from 38 eyes with UM immediately after enucleation and 22 eyes of cataract patients before cataract surgery. Aqueous samples of 0.05 mL from each eye were extracted using a standard sterilization procedure and immediately stored in a freezer at -80°C until assay. And they were tested within 6 months of collection.

Cytokines were measured via Luminex X-MAP technology using the Procarta Immunoassay kit (Panomics Inc, Fremont, CA), as described previously.^[12] Analyzed cytokines in this study included interleukin 6, 8 (IL-6, IL-8, respectively), interferon-inducible protein-10 (IP-10), placental growth factor1 (PIGF1), regulated on activation, normal T cell expressed and secreted (RANTES), monocyte chemoattractant protein-1 (MCP1), nerve growth factor-beta (NGF- β), epidermal growth factor (EGF), bFGF, and VEGF-A.

All data were analyzed using SPSS 20.0 for Mac (SPSS, IBM Corp, New York). Data are presented as the mean \pm standard deviation, and the normality of the distribution was assessed by the Shapiro–Wilk test. The nonparametric Mann–Whitney rank sum test and *t* test were performed to compare the differences between the study group and the control group. Two-tailed probability of less than .05 was considered to be a statistically significant difference.

3. Results

The study included 38 patients with UM in the study group and 22 patients with cataract in the control group. The mean age of the UM patients was 58.45 ± 12.36 years and that of the control group was 61.36 ± 7.62 years ($P = .23$). There was no significant difference in gender between 2 groups ($P = .19$). In the UM patients group, 47.7% were women, while that in the control group was 45.5% (Table 1). The tumor characteristics of UM patients were summarized in Table 2. Right eye was involved in 20 patients. 78.9% of the tumors were choroidal, while the rest were ciliochoroidal. Based on the AJCC 7th classification, tumor size categories were T3 in 29 patients (76.3%), and T4 in 9 patients (23.7%). Ten patients were in tumor stage II, while the rest were in tumor stage III. The mean largest basal tumor diameter was 15.8 mm (range, 12.5–25), and the tumor thickness was 10.2 mm (range, 8.1–17). No patient has extrascleral extension of tumor. All the patients were associated with serous retinal detachment.

Compared with cataract group, eyes with UM contained higher levels of all cytokines tested: IL-6 ($P = .006$), IL-8 ($P = .018$), IP-10 ($P = .004$), RANTES ($P = .008$), MCP-1 ($P = .018$), NGF- β ($P = .013$), EGF ($P < .001$), PIGF1 ($P = .01$), bFGF ($P = .016$), and VEGF ($P = .017$) (Table 3).

Table 1

Demographics of study population.

Characteristic	UM patients	Controls	<i>P</i> value
No.	38	22	
Female sex, no. (%)	17 (47.7%)	10 (45.5%)	.19*
Age (yr)			
Mean \pm SD	58.45 ± 12.36	61.36 ± 7.62	.23 [†]
Range	47–61	44–69	

Data are shown as mean \pm SD or number (%).

UM = uveal melanoma.

* χ^2 test.

[†] Mann–Whitney *U* test.

Table 2

Summary data on baseline ocular and tumor variables in patients with uveal melanoma.

Characteristic	No. (%)
Eye	
Right	20 (52.6%)
Left	18 (47.4%)
Tumor site	
Exclusively choroidal	30 (78.9%)
Ciliochoroidal	8 (21.1%)
TNM tumor size	
T3	29 (76.3%)
T4	9 (23.7%)
TNM stage	
II	10 (26.3%)
III	28 (73.7%)
Extrascleral extension of tumor	
Absent	38 (100%)
Largest basal diameter of tumor, mean \pm SD, [range], mm	15.8 ± 5.4 [12.5–25]
Thickness of tumor, mean \pm SD, [range], mm	10.2 ± 4.7 [8.1–17]

Data are shown as mean \pm SD or number (%).

$P = .02$), NGF- β ($P = .013$), EGF ($P < .001$), PIGF1 ($P = .01$), bFGF ($P = .016$), and VEGF ($P = .017$) (Table 3).

4. Discussion

In the current study, several angiogenic, inflammatory, and chemotactic cytokines are detected highly expressed in the aqueous humor of the UM eyes, when compared with the control eyes.

VEGF-A is a key pro-angiogenic factor associated with angiogenesis in numerous tumors.^[13] As previous studies reported,^[8,9] an abnormally high intraocular concentration of VEGF-A was also detected in eyes with UM in our study, probably producing by tumor cells and the tissues around.^[9] Increased serum VEGF was also detected in metastatic UM patients.^[14] Anti-VEGF therapy, such as bevacizumab, is currently used for the treatment of metastatic UM.^[15] We also

Table 3

Aqueous humor concentrations (pg/mL) (mean \pm SD) of cytokines in uveal melanoma patients and subjects undergoing routine cataract surgery (control group).

Cytokines	UM patients	Control	F value	<i>P</i> value
IL-6	37.58 ± 3.63	7.35 ± 1.37	1.168	.006 [†]
IL-8	50.67 ± 8.83	17.90 ± 9.02	8.413	.018*
IP-10	24.93 ± 1.13	1.52 ± 3.26	9.134	.004 [†]
PIGF1	12.63 ± 3.75	1.00 ± 0.85	7.113	.010*
RANTES	13.50 ± 0.21	3.35 ± 2.69	1.114	.008 [†]
MCP-1	16.81 ± 1.37	10.00 ± 8.81	5.376	.020*
NGF- β	19.23 ± 2.21	5.97 ± 3.16	7.564	.013*
EGF	1.93 ± 2.26	0.66 ± 0.22	8.778	<.001 [†]
bFGF	38.41 ± 7.58	21.67 ± 3.85	2.239	.016*
VEGF-A	6.91 ± 2.29	1.35 ± 3.66	1.437	.017*

Data are shown as mean \pm SD. Mann–Whitney *U* test.

bFGF = basic fibroblast growth factor, EGF = epidermal growth factor, IL = interleukin, IP-10 = interferon-inducible protein-10, MCP-1 = monocyte chemoattractant protein-1, NGF- β = nerve growth factor-beta, PIGF1 = placental growth factor1, RANTES = regulated on activation, normal T Cell expressed and secreted, UM = uveal melanoma, VEGF-A = vascular endothelia growth factor A.

* Indicated $P < .05$.

[†] Indicated $P < .01$.

found high levels of bFGF in aqueous of UM patients. Like VEGF-A, bFGF is also a potent pro-angiogenic cytokine, acting synergistically with VEGF-A to promote angiogenesis.^[16] Furthermore, we first demonstrated that the levels of PIGF1 elevated in the aqueous of UM patients in this study. PIGF1 is another important factor during retinal vascularization, belonging to the VEGF family. PIGF1 binds to VEGFR-1 and leads to angiogenesis.^[17] However, the role of PIGF in terms of tumor angiogenesis and tumor growth remains controversial. Some studies claim that PIGF is a cancer target promoting tumor angiogenesis and tumor growth, and anti-PIGF is useful for anti-cancer treatment,^[18–20] although other studies indicated that overexpression of PIGF suppresses tumor neovascularization and growth. Generally speaking, elevated angiogenic cytokines were detected in eyes with UM. As the metastasis of UM is mainly hematogenous, angiogenesis plays a crucial role in UM. Although antiangiogenic therapy has not yet been tested for the treatment of primary UM, it could be a potential choice for treatment in the future.

In this study, many inflammatory cytokines were also highly expressed in the aqueous of UM eyes. Generally, elevated IL-6, IL-8, sVCAM, IP-10, and RANTES were detected in the aqueous of uveitis patients.^[21] Inflammation has been proved playing important roles in tumor cells proliferation, angiogenesis, and metastasis. It disrupts the effective immune responses, and alters responses to chemotactic cytokines. IL-8 is a member of the chemokine family produced by a variety of cell types that activate and recruit polymorph nuclear leukocytes in acute and chronic inflammatory process. Previous study by Lattanzio et al^[22] indicated that IL-8 signal could be activated by the UM microenvironment as an alternative pro-angiogenic pathway beside VEGF. And it has been proven to trigger angiogenesis in vivo.^[23] Besides the tumor cells, normal cells around also produced cytokines like IL-6, IL-10, TGF- β , MIF, GM-CSF, and VEGF, involving immune responses.^[24] Elevations in IL-6 and IP-10 correlated with increased Treg infiltration.^[25] Jager et al^[26] defined macrophages with a high density in UM as “inflammatory phenotype,” usually accompanied with a high microvascular density and epithelioid cells. Macrophages differentiate to M2-type induced by IL-6 and IL-10, which have a tumor-promoting role associated with poor prognosis. Although the aqueous of UM-containing eyes showed a pattern of inflammatory phenotype, no diagnostic or prognostic value of individual cytokines was reported yet.

Recently, “tumor microenvironment” has attracted more attention in tumor growth and progression. A highlight in this field is the TAMs, which represent a predominant population of inflammatory cells in tumors. Chemo attractants such as MCP-1 could recruit TAMs from circulating monocytes into tissues.^[27] Significantly elevated MCP-1 was detected in our study, which was proved to be correlated with TAM in UM in a recent study.^[28] Besides the role of MCP-1 in chemotaxis of TAMs, it can also promote angiogenesis. And reduced angiogenesis and tumor growth were observed in a mouse model when MCP-1 was inhibited.^[29] Epidermal growth factor receptor (EGFR) has been detected in many cancers and considered to be important in tumor growth. Expression of EGFR has been reported in 12.5% to 29.2% UM patients,^[30,31] while the elevated vitreal EGF levels were also detected in some UM patients. And in our study, the expression of EGF in aqueous of UM patients is significantly higher than the control group. As previous study reported, the expression of EGFR and EGF were correlated with mitosis rate and scleral invasion, respectively;^[31] which indicated that EGF-EGFR signal seems to be a novel therapeutic target for UM. NGF-

β was another elevated cytokine in our study, which was found to modulate the tumor cells migration, inflammatory response and promote tumor progression.^[32–34] Expression of NGF-R was found in primary and metastatic melanoma lesions, associated with a poor prognosis.^[35]

Certainly, there are still some limitations in our study. The relations between cytokines and clinicopathological parameters of tumor could not be analyzed because of the incomplete clinical data. Previous studies reported that elevated cytokines probably correlate with tumor size and the severity of retinal detachment; however, no correlation was found in this group probably caused by the selection bias and small sample size. Meanwhile, it is unclear of the causal relationship between tumor and elevated cytokines. Thus, further studies are necessary to clarify the role of cytokines in pathogenesis of UM.

5. Conclusion

In conclusion, this study demonstrated that eyes with UM contain higher aqueous concentrations of angiogenic, inflammatory, and chemotactic cytokines, which indicate that these cytokines were closely interrelated with the disease and could be potential targets for treatment.

Author contributions

YC, JF, and XZ contributed to conception, designed the study, collected and analyzed data, as well as drafted manuscript and submission. JL contributed to protocol assessment, data interpretation, and manuscript revising. All authors read and approved the final manuscript.

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