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Expression of molecular markers of peripheral blood lymphocyte activation before and after eye-preserving treatment for choroidal melanomas of various stages

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Purpose. *To assess the expression of molecular markers of peripheral blood lymphocyte activation (CD7+, CD54+, CD95+) before and after eye-preserving treatment for T1 to T3 choroidal melanoma (CM).*

Material and Methods: *Expression of molecular markers of peripheral blood lymphocyte activation (CD7+, CD54+, CD95+) was examined before and after eye-preserving treatment for T1 to T3 CM. The major study group (group 1) consisted of 25 patients (14 (56.0%) women and 11 (44.0%) men; mean age (standard deviation (SD)), 51.7 (16.6) years) who received the eye-preserving treatment (transpupillary thermotherapy (TTT (delivered using the developed methodology) combined with strontium-90/yttrium-90 (90Sr/90Y) brachytherapy (BT)) for moderate to large (T1-T3) CM (tumor base, 3.1 to 15 mm; tumor thickness, 3.1 to 9.0 mm). The control group (group 2) consisted of 16 patients (12 (75.0%) women and 4 (25.0%) men; mean age (SD), 55.4 (11.2) years) who received TTT only for small (T1) CM (tumor base, ≤ 12 mm; tumor thickness, ≤ 3 mm).*

Results: *The expression of markers CD7+, CD54+, and CD95+ before treatment for T1-T3 CM was higher than before treatment for small T1 CM, but the difference was significant only for the absolute expression of CD7+ (p = 0.0008).*

After TTT for small T1 CM, a statistically significant increase was observed only in the absolute and percentage expression of CD7+ (p = 0.04 and p = 0.0000, respectively) and the absolute expression of FAS-ligand CD95+ (p = 0.05). Additionally, the level of CD54+ increased after treatment, but not significantly. Absolute expression levels of these molecular markers decreased after TTT plus BT, but not significantly. Additionally, the percentage expression of CD7+ decreased statistically significantly (p = 0.02), and the percentage expression of CD95+ decreased statistically insignificantly (p = 0.06). After eye-preserving treatment for CM, there were significant differences between the two groups in the absolute and percentage expression of FAS-ligand CD95+ (p = 0.003 and p = 0.000, respectively) and CD54+ (p = 0.03 and p = 0.000 respectively), with these levels being lower in group 1.

Conclusion: *Expression of molecular markers of peripheral blood lymphocyte activation (CD7+, CD54+, CD95+) increased with tumor progression in patients with CM. The expression of the markers increased after TTT only for small T1 CM, and decreased after TTT plus BT (90Sr/90Y) for T1-T3 CM, likely due to the effect of radiotherapy.*

Key words:

choroid, immunology, radiotherapy, transpupillary thermotherapy, molecular markers, ophthalmic oncology, choroidal melanoma

Introduction

The human immune system actively responds to the development of tumor process and pathogenetic mechanisms of progressive tumor growth associated with the interaction of immunocompetent and tumor cells [1-6].

Functional activity of immunocompetent cells of the body in the form of activation of receptors of peripheral blood lymphocytes is important for antitumor action and is manifested by the expression of various functional molecules on the surface of these lymphocytes [7, 8]. Prior to treatment, patients with small (i.e., tumor base ≤ 12 mm and thickness ≤ 3 mm) T1 choroidal melanoma (CM) were found to have elevated expression of molecular markers of peripheral blood lymphocyte activation (CD7+, CD54+, CD95+) compared to healthy controls [9]. In addition, the expression of molecular markers of peripheral blood lymphocyte activation, especially the expression of the co-stimulatory molecule inducing cytokine secretion (CD7+) and apoptotic lymphocyte activity due to an increased share of FAS-ligand CD95+, was found to increase within the course of transpupillary thermotherapy (TTT) for the small (T1) CM [10].

Therefore, research on the molecular mechanisms underlying protection against tumor growth in patients with CM is important in ophthalmic oncology.

The purpose of this study was to assess the expression of molecular markers of peripheral blood lymphocyte activation (CD7+, CD54+, CD95+) before and after eye-preserving treatment for T1 to T3 CM.

Material and Methods

A monoclonclonal antibody panel was used to examine the expression of molecular markers of peripheral blood lymphocyte activation (CD7+, CD54+, CD95+) before and after eye-preserving treatment for T1 to T3 CM. The major study group (group 1) consisted of 25 patients (14 (56.0%) women and 11 (44.0%) men; mean age (standard deviation), 51.7 (16.6) years) who received the eye-preserving treatment (TTT (delivered using the developed methodology [11]) combined with strontium-90/yttrium-90 (90Sr/90Y) brachytherapy (BT)) for moderate to large (T1-T3) CM (tumor base, 3.1 to 15 mm; tumor thickness, 3.1 to 9.0 mm). The control group (group 2) consisted of 16 patients (12 (75.0%) women and 4 (25.0%) men; mean age (standard deviation), 55.4 (11.2) years) who received TTT only for small (T1) CM (tumor base, ≤ 12 mm; tumor thickness, ≤ 3 mm).

Five-ml fasting venous blood samples were taken to assess levels of peripheral lymphocyte activation markers before and after a course of treatment [12].

Tumor stage was determined using the 2009 American Joint Commission on Cancer Tumor, Node and Metastases (TNM) classification scheme [13].

Abdominal cavity ultrasound and chest computed tomography found no metastasis in any patient at baseline.

The statistical power was analyzed using G*Power 3.1 software. Mean and SD values were calculated. One-way analysis of variance (ANOVA) and post-hoc Fisher's test were used for statistical analysis. P values ≤ 0.05 were considered significant.

Results

Table 1 shows the expression of molecular markers of peripheral blood lymphocyte activation (CD7+, CD54+, CD95+) before and after eye-preserving treatment for T1-T3 CM.

The expression of molecular markers of peripheral blood lymphocyte activation before treatment for T1-T3 CM was higher than before treatment for small T1 CM, but the difference was significant only for the absolute expression of CD7+ (p = 0.0008) (Table 1).

In group 2, after TTT for small T1 CM, a statistically significant increase was observed only in the absolute and percentage expression of the costimulatory molecule inducing cytokine secretion (CD7+) (p = 0.04 and p = 0.0000) and the absolute expression of FAS-ligand CD95+ (p = 0.05) that effects the apoptotic lymphocyte activity. Additionally, the level of CD54+ also increased after treatment, but not significantly.

In group 1, absolute expression levels of these molecular markers decreased after the eye-preserving treatment (TTT plus BT), but not significantly. Additionally, the percentage expression of CD7+ decreased statistically significantly (p = 0.02), and the percentage expression of CD95+ decreased statistically insignificantly (p = 0.06).

Moreover, after eye-preserving treatment for CM, there were significant differences between the two groups in the absolute and percentage expression of FAS-ligand CD95+ (p = 0.003 and p = 0.000, respectively) and CD54+ (p = 0.03 and p = 0.000 respectively), with these levels being lower in group 1.

Table 1. Expression (mean [standard deviation]) of molecular markers of peripheral blood lymphocyte activation ($CD7^+$, $CD54^+$, $CD95^+$) before and after eye-preserving treatment for choroidal melanomas of various stages

Monoclonal antibodies	Group 1		p_{1-2}	Group 2		p_{3-4}	p_{1-3}, p_{2-4},
	Before treatment n =25	After treatment n = 25		Before treatment n =16	After treatment n =16		
	1	2		3	4		
$CD7^+$ (cell/μL)	529.9 (128.5)	483.3 (124.5)↓	0.20	347.8 (192.2)	553.5 (189.0)↑	0.04	p_{1-3}=0.0008 p_{2-4}= 0.15
$CD7^+$ (%)	25.9 (2.3)	23.8 (3.8)↓	0.02	25.8 (3.0)	26.0 (5.5)↑	0.0000	p_{1-3}=0.9, p_{2-4}= 0.14
$CD54^+$ (cell/μL)	527.5 (157.5)	510.1 (150.8)↓	0.69	458.1 (220.2)	643.3 (232.4)↑	0.10	p_{1-3}=0.25, p_{2-4}= 0.03
$CD54^+$ (%)	26.1 (2.5)	25.3 (2.4)↓	0.25	24.9 (8.3)	30.7 (4.2)↑	0.12	p_{1-3}=0.5, p_{2-4}=0.000
$CD95^+$ (cell/μL)	527.9 (139.0)	499.6 (136.9)↓	0.47	501.1 (198.9)	705.9 (281.3)↑	0.05	p_{1-3}=0.61, p_{2-4}= 0.003
$CD95^+$ (%)	25.7 (2.6)	24.4 (2.2)↓	0.06	27.9 (8.0)	33.6 (7.1)↑	0.10	p_{1-3}=0.21, p_{2-4}= 0.000

Note: P, Fisher's exact P-value; n, number of patients; ↑, arrow denoting an increase in the parameter after treatment; ↓, arrow denoting a decrease in the parameter after treatment

Discussion

Higher pre-treatment expression levels of molecular markers CD7+, CD54+ and CD95+ in patients with T1-T3 CM compared to patients with small T1 CM indicate that lymphocytes (the cells important for immune response regulation) become activated as the tumor develops. The CD7+ marker may contribute to an increase in the T-cell subsets producing cytokines, low molecular weight proteins that endogenously regulate intercellular interaction of all the components of the immune system and inflammatory hematopoiesis as well as intersystem interactions. These T-cell subsets impact the differentiation, functional activity, stimulation of the growth and survival of tumor cells, and a 40-percent increase in the number of these T-cells in the presence of tumor development may contribute to tumor progression and reduction in antitumor immune response [1, 2, 8].

The increase in the expression of FAS-ligand CD95+ in patients with moderate to large (T1-T3) CM indicates the apoptotic lymphocyte activity leading to the inhibition of excessive immune response and contributes to immune homeostasis [1, 2].

Activation of intercellular adhesion is important, and CD54+ is involved in this process. Intercellular adhesion molecule 1 (ICAM-1) is a member of the immunoglobulin superfamily and a transmembrane protein that is expressed on the surface of several cell types and impacts their intercellular adhesive interaction. Activation of intercellular adhesion plays an important role in the immune system, especially in leukocyte adhesion to the endothelium and transendothelial migration. ICAM-1 is involved in the immunological synapse between lymphocytes and antigen-presenting cells. An increased expression of CD54+ is believed to be associated with the development of pathological inflammation in various disorders (e.g., cancer) [14–17].

Given that ICAM-1 has been shown to be expressed by retinal and choroidal endothelial cells in eyes of patients with non-infectious uveitis of the anterior and posterior segments [18–20], it may be hypothesized that the increase in CD54+ expression in the development of the tumor (e.g., melanoma) in the choroid occurs due to a negative effect of secondary inflammation that accompanies tumor development and increases with tumor progression and in response to treatment, which we observed in our patients.

Conclusion

Expression of molecular markers of peripheral blood lymphocyte activation (CD7+, CD54+, CD95+) increased with tumor progression in patients with CM. In group 2, due to the effect of TTT on small T1 CM, the absolute and percentage expression of the costimulatory molecule inducing cytokine secretion (CD7+) increased 1.6-fold ($p = 0.04$ and $p = 0.0000$, respectively), and the absolute expression of FAS-ligand CD95+ ($p = 0.05$) effecting apoptotic lymphocyte activity increased 1.4-fold. In group 1, after TTT plus BT (90Sr/90Y) for moderate to large (T1-T3) CM, absolute expression of these markers in blood tended to decrease. Additionally, the percentage expression of CD7+ decreased statistically significantly ($p = 0.02$), and the percentage expression of CD95+ decreased statistically insignificantly ($p = 0.06$). The reduction in immunological characteristics in patients after combinatory treatment for CM could be associated with the effect of radiotherapy.

Investigation of the mechanisms of interplay between expressions of molecular markers (namely, lymphocyte molecular markers inducing cytokine secretion and apoptotic activity) is helpful for determining immune response to tumor progression and tumor response to treatment. This will enable identifying among these molecules potential markers for assessing and monitoring tumor progression and metastasizing and evaluating tumor treatment efficacy.

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Disclosures

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Author's contribution. *DAD: Data curation and analysis, Writing – original draft preparation; IVT: Data curation and analysis, Database development SIP: Conceptualization and project administration, Data interpretation, Writing – review and editing; LMV: Analysis of investigation results, Writing – review and editing; MBM: Investigation, Data analysis; OVB: Investigation, Data analysis. All authors read and approved the final version of the manuscript. All authors analysed the results and approved the final version of the manuscript to publication.*

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Data Availability Declaration: *All the data obtained or examined during this study has been incorporated into this published article.*

Abbreviations: *BT, brachytherapy; CM, choroidal melanoma; PC, photocoagulation; TTT, transpupillary thermotherapy*