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## **Angiogenesis and screening in uveal melanoma**

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## Heat shock protein expression in the eye and in uveal melanoma

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## ABSTRACT

### Purpose

Expression of heat shock proteins (HSPs) is of prognostic significance in several tumor types, whereas HSPs may also have clinical use as stimulators in tumor vaccination. HSP expression levels were determined in normal eyes and in uveal melanoma and tested whether HSPs expression was associated with prognostic parameters in the uveal melanoma.

### Methods

Expression of HSP27, HSP70, HSP90, and glycoprotein96 (GP96) were determined on paraffin-embedded and frozen sections from seven healthy eyes, 20 primary uveal melanomas without prior treatment, and 18 uveal melanomas after prior treatment. HSP expression was determined by alkaline phosphatase-anti-alkaline phosphatase (APAAP) immunohistochemistry, using appropriate monoclonal antibodies and scored semiquantitatively. Expression of HSPs was validated on retinal tissue of a normal eye and in two uveal melanoma cell lines by Western blot analysis.

### Results

Expression of HSPs was observed in epithelial and pigment cells of the normal eyes. In uveal melanoma, the level of expression of HSPs varied. Expression of HSP27 and GP96 was noted in more than 30 of 38 uveal melanomas (with, respectively, a mean of 66% and 53% positive cells). HSP70 and HSP90 were expressed in 6% of tumor cells. The amount of expression of any of the HSP types was not significantly associated with known prognostic factors. There was not a significant difference in expression of the HSPs between uveal melanomas with or without any type of prior treatment.

### Conclusions

In this study, expression of HSPs in uveal melanoma is not correlated with known histopathologic prognostic factors. The high expression of GP96 indicates that this protein is a potential vector in tumor vaccination in patients with large uveal melanomas.

## INTRODUCTION

Uveal melanoma is the most common primary intraocular neoplasm in adults.<sup>1</sup> It has an incidence in the white population of 0.6 to 0.8 per 100,000. Although only 2% to 3% of patients present with detectable metastases at the time of diagnosis, over 30% of patients will have metastases within 5 years.<sup>1</sup> The 10-year survival is approximately 60%. The prognosis, once metastases have formed, is very poor. Only 15% of patients survive the first year after diagnosis of metastasis (with a mean survival rate of 5–8 months).<sup>2</sup> Development of an effective treatment of metastatic uveal melanoma is therefore required. The interest in the immune system as a tool for such a treatment is growing, and heat shock proteins (HSPs) might be a tool in future melanoma immunotherapy.

HSPs are abundant soluble intracellular proteins.<sup>3</sup> Although these proteins were first discovered after a heat shock, they are also upregulated by other forms of stress.<sup>4</sup> The main function of these adenosine triphosphate (ATP)-dependent proteins is a chaperone function. They associate with proteins or peptides in a way that modifies the destiny and function of the latter.<sup>5</sup>

Cytoplasmic HSPs are involved in the caspase-3 and -9 apoptosis pathway. HSP27 and -70 inhibit the formation of a functional proteasome, thus inhibiting proteolysis of the cell.<sup>6,7,8</sup> HSPs help to prevent immediate apoptosis of the cell after a shock, allowing repair mechanisms to function and giving the cell a chance to survive. Furthermore, HSPs chaperone denatured proteins in the stressed cells, giving the proteins time to reestablish their normal structure and escaping the risk of cell death. As such, HSPs have become of interest in radiotherapy and in radiation therapy.

HSPs also have a chaperone function in nonstressed cells—that is, in the antigen-presentation pathway by binding antigenic peptides. The cytoplasmic isoforms of HSP70 and HSP90 can bind to antigenic peptides and bring them to the endoplasmic reticulum. Once in the endoplasmic reticulum, the antigenic peptides are mounted on the HLA-class I molecules with the support of the glycoprotein GP96 (GRP94), the endoplasmic isoform of HSP90.<sup>9</sup> HSPs also interact with antigen-presenting cells through CD91 and other receptors, eliciting a cascade of events including representation of HSP-chaperoned peptides by the major histocompatibility complex (MHC).<sup>10</sup>

HSP70, GP96, and sometimes HSP90 are expressed on the surface of normal and tumor cells at very low concentrations. Surface HSP expression is increased after heat shock of normal cells, such as cutaneous epithelium<sup>11</sup> and retinal pigment epithelium<sup>12</sup> and in tumor cells, such as uveal melanoma cells.<sup>13</sup> An increase of HSPs on the cell surface has also been associated with an increase of the level of MHC class I antigens and higher immunogenicity.<sup>14</sup>

Immunotherapy based on HSPs has been proposed by Ishii et al.,<sup>15</sup> by isolating the HSP-antigen peptide complexes through a chromatography step from the primary tumor, and reinjecting them into the patient. Injecting the antigens together with the HSPs caused the antigens to be quickly absorbed by antigen-presenting cells and presented to the im-

une system. This mixture, extracted from the patients' primary tumor, contains all the antigenic peptides from this specific tumor, evoking a tumor-specific immune reaction. Most experiments with this therapy are performed with HSP70 and GP96 immunization in renal cancer, colorectal cancer, gastric cancer, and malignant melanoma.<sup>16</sup>

The primary purpose of the present study was to investigate the expression of HSPs in the normal human eye and in the uveal melanoma and to investigate the prognostic value of HSPs in eyes with uveal melanoma. Second, because the expression of HSPs is increased by stress, such as radiation or radiotherapy, we evaluated the expression of HSPs on stressed tumors, by examining an untreated and treated group. Third, we investigated whether the expression of HSP70, HSP90, or GP96 is sufficient in light of the possible use of these proteins in immune-based treatment of uveal melanoma.

## MATERIALS AND METHODS

### Patients and Tumors

Between 1995 and 2001, tumor specimens were obtained immediately after enucleation of 38 eyes of 38 patients with uveal melanoma. The specimens were immediately fixed in formaldehyde 4.0% and embedded in paraffin for 48 hours. In 20 tumors, enucleation was the primary procedure; in 10, uveal melanomas were enucleated after previous ruthenium plaque radiotherapy, and in 5 experimental pre-enucleation transscleral radiotherapy (TSTT) was performed.<sup>17</sup> Three eyes had undergone proton beam treatment before enucleation. Absence of distant metastasis at the moment of enucleation was determined by abdominal ultrasound, thorax x-ray, and liver serum tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], -glutamyltransferase [GGT], and lactate dehydrogenase). The age of the patients ranged from 44 to 86 years (average, 62 years) at enucleation. Twenty-two (58%) women and 16 (42%) men were included. Of the eyes studied, 22 (58%) were right eyes and 16 (42%) were left eyes.

Each specimen was processed for histopathologic examination by an ophthalmic pathologist. The tumors were classified according to cell type and tumor localization. Largest tumor diameter and height of the tumor were measured in millimeters, and the number of mitoses was counted in 15 high-power fields with a magnification of  $\times 320$ . Vascular patterns were classified in accordance with the scheme of Folberg et al.<sup>18</sup> Specimens were obtained in accordance to the World Medical Association (WMA) Declaration of Helsinki and the Medical Ethics Committee of Leiden University Medical Center.

To investigate the expression of HSPs in normal eyes, paraffin blocks of two eyes of patients without known ocular problems (enucleated because of intraorbital glioma) and five frozen sections of normal retinas were used. Eyes of five persons without known eye disease were obtained from the Corneabank Amsterdam (The Netherlands), after removal of corneal buttons for transplantation. Enucleation was performed within 2 hours after death. Intact eyes were snap frozen in isopentane and stored at  $-80^{\circ}\text{C}$  until used. Tissue blocks of the posterior half of the frozen human globes were cut according to a standard protocol. Air-dried serial cryostat sections ( $10\ \mu\text{m}$  thick) of one tissue block containing midperipheral and central retina of one eye of each patient were fixed in cold acetone for 10 minutes and stained by an indirect immunoperoxidase procedure.

### Immunohistochemistry

Staining for HSPs for all eyes (tumor and nontumor) was performed by the alkaline phosphatase-anti-alkaline phosphatase (APAAP) method.<sup>19</sup> Paraffin-embedded sections ( $4\ \mu\text{m}$ ), mounted on slides coated with aminopropyltriethoxy-silane (APES; Sigma-Aldrich, St. Louis, MO), were deparaffinized in xylene (two times, 10 minutes each) and ethanol 99% (two times, 5 minutes each). The endogenous peroxidase activity was blocked by incubating the slides with methanol/ $\text{H}_2\text{O}_2$  0.3% for 20 minutes. After the slides were washed, antigen retrieval was performed by boiling in citrate buffer (Dako, Glostrup, Denmark) for 10 minutes. After the slides were washed again in phosphate-buffered saline (PBS), they were incubated with the first antibody for 1 hour. Mouse anti-HSP27 mAb (Stressgen, Victoria, British Columbia, Canada) clone G3.1 (dilution 1:400); mouse anti-HSP70 mAb (Stressgen) clone C92F3A-5 (dilution 1:200); mouse anti-HSP90 mAb (Stressgen) clone AC88 (dilution 1:400) and rat anti-GRP94 mAb (Stressgen) clone 9G10 (1:200) were used. As a negative control a monoclonal mouse IgG1 primary antibody (clone DAK-GO1; Dako) and monoclonal rat IgG2a primary antibody (clone LO-DNP-16; Serotec, Oxford, UK) were used. Slides were then incubated with secondary poly-AP goat anti-mouse antibody (Immunovision Technologies, Springdale, AZ) or with goat anti-rat IgG-AP (Stressgen) for 1 hour. The slides were labeled with fast red (Scytek, Logan, UT) in naphthol-phosphate buffer (Scytek) and levamisole 50 mM. After 20 minutes, this reaction was stopped in distilled water. Slides were counterstained with Mayer's hematoxylin and finally embedded in Kaiser's glycerin.

Sections of colon carcinoma (GP96), gastric carcinoma (HSP70), and normal skin (HSP27 and HSP90) were used in positive control experiments for the expression of HSPs. Staining of the uveal melanoma sections was evaluated by two independent observers who counted the percentage of stained tumor cells. In case of discrepancy, slides were reevaluated until consensus was reached.

### Western Blot

To confirm the staining results, Western blot analysis of HSPs was performed on normal human retinal tissue (obtained from the Corneabank, Amsterdam) and on two primary uveal melanoma cell lines: Mel-202 and OCM-1. Cell line Mel-202 was a generous gift of Bruce R. Ksander (Schepens Eye Institute, Harvard Medical School, Boston, MA) and cell line OCM-1 was kindly provided by June Kan-Mitchell (University of California, San Diego, CA).

Retinal tissue was dissected, homogenized in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) buffer and boiled for 4 minutes. Western blot was performed using the mentioned monoclonal antibodies and appropriate secondary antibodies. Protein bands were visualized using a chemiluminescence substrate kit (Supersignal; Pierce, Rockford, IL).

### Statistics

Differences between treated and untreated groups were compared with a Student's *t*-test. Correlations between the expression of the different HSPs and cell type, pigmentation grade, vascular pattern classification, and mitosis grade were analyzed with an ANOVA test. For statistical analysis of the vascular patterns, a classification was made between tumors with loops and/or networks (Folberg classes 8 and 9) and tumors without them (classes 1–7). Correlations between expression of HSPs, tumor diameter, and height were determined with the bivariate Pearson correlation. All statistical processing was performed on computer (SPSS ver. 10.0; SPSS Inc, Chicago, IL).

## RESULTS

### Expression of HSPs in the Human Eye

In normal eyes, especially epithelial cells and pigmented cells were stained. All HSPs were expressed in the RPE, and only HSP27 and HSP90 stained both the inner and outer plexiform layers (Table 1). Expression of HSP27, HSP70, and GP96 was also observed in the ganglion cell layer. The choroid clearly expressed HSP27 and GP96, and only weakly expressed HSP70 (Fig. 1A). Expression of HSP27 occurred in both the corneal epithelium and endothelium (Fig. 1B). Other HSPs were not expressed by the corneal endothelium. Both retinal and scleral blood vessels expressed HSP27. Expression of HSPs was confirmed by Western blot.

	HSP27	HSP70	HSP90	GP96
<b>Cornea</b>				
Epithelium	+++	+	+	+
Stroma	-	-	-	-
Endothelium	+	-	-	-
<b>Iris</b>				
Posterior pigment Epithelium	++	+	+	-
Iris sphincter	+	-	-	-
<b>Lens</b>				
Epithelium at equator	+	-	-	-
<b>Ciliary body</b>				
Epithelium and pigment layer	++	++	+	++
Eiliary muscle	++	++	-	-
Choroid	+++	+	-	+
<b>Retina</b>				
Ganglion cell layer	++	+	-	+
Inner and outer cellular layer	+	-	+	-
RPE	+	+	+	+
<b>Sclera</b>				
Scleral vessel endothelium	+	-	-	-
<b>Optic nerve</b>				
	-	-	+	+

Table 1. Expression of HSPs in the Normal Eye

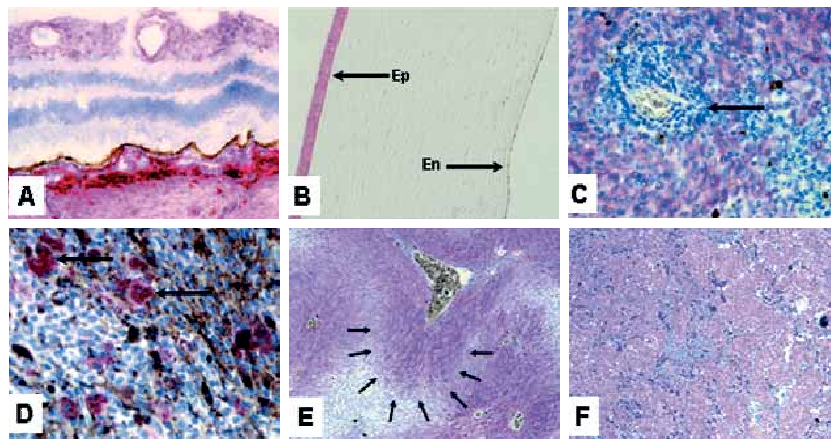


Figure 1.

**Uveal Melanomas without Prior Treatment**

HSP27 was expressed in all tumor tissues, with a high percentage of cells stained (average mean ± SEM, 71.5% ± 5.4%; Table 2 ). A fine granular cytoplasmic staining was observed in all positive tumor cells. Blood vessels within the tumor were not stained (Fig. 1C) .

Patient	Age (y)	Eye	Cell Type*	Pigm.	Dia. (mm)	Ht. (mm)	Mitosis †	Vascular Patterns ‡	HSP,			
									27	70	90	96
1	57	OS	1	2	12	9	1	8	40	10	0	95
2	61	OD	1	2	14	8	3	8	30	2	5	60
3	63	OS	2	1	14	6	8	9	85	0	5	60
4	48	OD	2	1	20	6	5	9	100	0	0	100
5	53	OD	2	2	14	9	4	7	50	0	5	50
6	47	OD	2	3	15	7	3	7	60	30	40	40
7	41	OD	2	2	13	5	3	9	60	5	0	60
8	69	OD	2	1	8	6.5	2	8	95	0	0	50
9	84	OS	2	2	13	5	4	7	55	10	8	85
10	71	OS	2	2	15	7	3	8	95	5	0	0
11	49	OD	2	2	14	9	5	2	80	15	10	5
12	75	OS	2	1	17	12	8	9	30	0	0	30
13	45	OS	3	1	14	6	3	7	100	15	5	0
14	86	OS	3	2	11	9	2	7	100	5	5	65
15	57	OS	3	1	12	9	3	8	50	5	0	60
16	56	OS	3	1	12	6	3	6	85	1	0	5
17	50	OD	3	2	16	8	5	7	70	10	40	75
18	84	OD	3	1	9	6	4	8	60	2	1	100
19	53	OS	3	2	12	10	2	9	90	10	5	95
20	44	OD	3	1	11	11	5	9	95	5	5	95
Mean	58.7				13.3	7.7	3.8		71	6	7	56
SEM	2.9				0.6	0.4	0.4		5	2	3	

Age is that at enucleation. Dia., diameter; Ht, height; Pigm., pigmentation.  
 \* : 1, Epitheloid; 2, mixed; 3, spindle.  
 † : 1, Slight ( 50% of cells); 2, moderate ( 50% and 100%); 3, severe (100%).  
 ‡ : Number of mitoses per 15 fields of microscopic examination (magnification, 320)  
 § : 1, Normal; 2, silence; 3, straight; 4, para; 5, para cross; 6, arcs; 7, arcs bran; 8, loops; 9, networks. According to the Folberg classification.<sup>18</sup>  
 Highest number present in tumor.  
 || : Percentage of cells staining for HSPs.

Table 2. Expression of HSPs and Histopathologic Parameters in Uveal Melanomas without Prior Treatment

HSP70 was expressed in 15 of the 20 tumors. The staining of HSP70 was less intense; only up to 30% (average  $6.5\% \pm 1.6\%$ ) of tumor cells were positive (Fig. 1D). HSP90 was expressed in 12 of 20 tumors and there were only a few positive cells (0%–40%), with a mean staining of  $6.7\% \pm 2.6\%$  (Fig. 1E). Staining for the GP96-antibody was found in 18 of 20 tumors with staining of 0% to 100% (average,  $56.5\% \pm 7.6\%$ ; Fig. 1F). Expression of HSP70 correlated with that of HSP90 ( $P < 0.01$ ). The expression of the different HSPs did not correlate with a particular cell type. HSP expression outside the tumor was similar to that in normal eyes.

No correlation could be established between expression of HSP and the prognostic factors mitotic rate ( $P = 0.114, 0.738, 0.844,$  and  $0.194$  for HSP27, -70, and -90 and GP96, respectively), cell type ( $P = 0.060, 0.995, 0.872,$  and  $0.481$ ), tumor height ( $P = 0.337, 0.878, 0.969,$  and  $0.743$ ), basal tumor diameter ( $P = 0.697, 0.666, 0.257,$  and  $0.526$ ), or vascular patterns ( $P = 0.700, 0.868, 0.940,$  and  $0.500$ ). There was, however, a significantly positive correlation between HSP70 and high pigmentation ( $P < 0.01$ ), and also between HSP90 and high pigmentation ( $P < 0.01$ ).

The expression of HSPs in uveal melanoma cells was confirmed by Western blot analysis (Fig. 2) on two uveal melanoma cell lines. The blot results of the cell lines confirmed immunohistochemical results of cytopins of these cell lines.

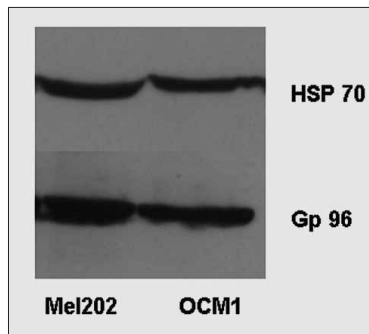


Figure 2. Western blot analysis of two uveal melanoma cell lines for HSP70 and GP96.

All HSPs were demonstrated on the uveal melanoma cell lines and normal human retina.

### Uveal Melanomas with Prior Treatment

Evaluation of the uveal melanomas after previous treatment showed that large parts of the tumors were necrotic and not stained with the antibodies (Table 3). The necrotic zones were not taken into account for determining the percentages of stained cells. Globally, the results for treated tumors were comparable with those of nontreated tumors (Fig. 3). The results for the different subgroups are shown in Figure 4. As in the nontreated eyes, blood vessels in the tumor did not stain.

Patient	Age (y)	Eye	Therapy *	Cell Type	Pigm.	Dia. (mm)	Ht. (mm)	Mitosis	HSP			
									27	70	90	96
1	49	OD	1	1	3	7	2.5	0	100	30	0	100
2	64	OD	1	1	1	7.7	1.9	2	100	0	0	100
3	64	OS	1	1	1	20	1.5	8	100	5	5	100
4	57	OD	1	2	1	20	1.5	0	90	0	0	100
5	70	OD	1	2	1	8	1	0	60	5	5	0
6	73	OS	1	2	2	17	1.7	1	30	20	40	25
7	46	OS	1	2	1	7	3	1	100	5	0	100
8	76	OD	1	3	1	9.2	0.6	0	0	0	0	0
9	78	OD	1	3	2	6.9	1.3	0	0	0	5	0
10	77	OD	1	4	3	7	2	0	0	5	0	0
11	77	OD	2	2	1	11	6	0	0	5	5	5
12	53	OS	2	2	2	16	7	10	90	0	0	70
13	57	OD	2	2	2	13	9	0	80	0	30	0
14	56	OD	2	3	2	10	9	0	30	0	0	100
15	82	OS	2	4	3	15	13	0	100	0	0	100
16	57	OD	3	1	1	11	1		60	0	0	60
17	57	OD	3	2	1	8	1	1	60	0	0	0
18	45	OS	3	3	2	12	6	2	70	10	5	30
Mean	63.3					11.4	3.8	1.7	59	5	5	49
SEM	2.7					1	0.9	0.8	9	2	3	11

Age is that at enucleation. Dia., diameter; Ht, height; Pigm., pigmentation.

\* : 1, Ruthenium application; 2, TSTT; 3, protonbeam irradiation.

The remaining data and abbreviations are as in Table 2.

Table 3. Expression of HSPs and Histopathologic Characteristics in Uveal Melanomas That Received Another Therapy Prior to Enucleation

Again, a correlation was found between HSP70 and pigmentation grade ( $P < 0.05$ ). However, in contrast with the untreated group, there was no relation between pigmentation and HSP90. The expression of HSPs was not significantly related with any other histopathologic characteristics, such as mitotic rate ( $P = 0.506, 0.964, 0.861, \text{ and } 0.540$  for HSP27, -70, and -90 and GP96, respectively), cell type ( $P = 0.135, 0.595, 0.406, \text{ and } 0.556$ ), tumor height ( $P = 0.418, 0.478, 0.762, \text{ and } 0.275$ ), or basal tumor diameter ( $0.172, 0.809, 0.192, \text{ and } 0.056$ ). The differences between the percentage of positively stained cells in the treated and untreated groups and between the different treatments were not significant.

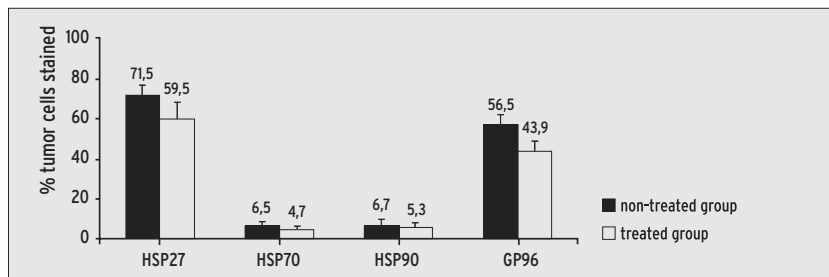


Figure 3. Expression of the HSPs in percentage of tumor cells (on or above the bars) stained for the nontreated ( $n = 20$ ) and the treated group ( $n = 18$ ). Error bar, SEM.

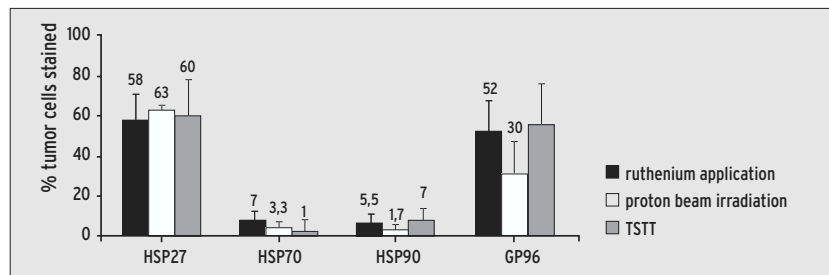


Figure 4. Expression of the HSPs in percentage (on or above the bars) of tumor cells stained for the different groups with treatment before enucleation: ruthenium application ( $n = 10$ ), TSTT ( $n = 5$ ), and proton beam irradiation ( $n = 3$ ). Error bars, SEM.

## DISCUSSION

Our study is the first report of the expression of HSPs in the whole human eye. The results show that HSPs are expressed in the epithelium and pigmented cells of the human eye, as reported earlier in animals and human cell lines, although there are some differences. Both in humans and in mice,<sup>20</sup> expression of HSP27 was found in the normal RPE of almost all eyes. HSP70, however, was only slightly expressed in the human RPE as indicated previously in RPE cell lines,<sup>21</sup> in contrast to abundant expression in mice.<sup>22</sup> Other results confirm the expression of HSP90 by the RPE.<sup>12</sup> A light staining of corneal endothelial cells with HSP70 and HSP90 has been described<sup>23,24</sup> but could not be confirmed in our results. The expression of GP96 has been demonstrated in cutaneous melanoma cell lines<sup>25</sup> and has now been confirmed in primary uveal melanoma.

In the literature,<sup>26-36</sup> heat shock protein expression patterns are of prognostic significance in many tumors, such as breast cancer, prostate cancer, small cellular lung carcinoma, and others. However, in our study, there was no correlation of expression of HSP27, -70, or -90 or GP96 with such important prognostic factors as basal tumor diameter, tumor height, cell type, mitosis rate, and tumor localization. That high levels of HSPs are associated with a good prognosis in some malignancies and with a poor prognosis in others could indicate that HSPs have different roles in different tissues or that there are other elements present in some malignancies that can override or bypass any effect of the HSPs. It is a fact that several pathways exist for the transport and final presentation of antigenic proteins on the HLA class I molecules (e.g., TAP-1, TAP-2, and HSP70 pathway for transport of peptides to endoplasmic reticulum). This may explain the lack of prognostic importance of HSPs in our study. Furthermore, no higher expression of HSPs were found in the tumors treated with TSTT, ruthenium therapy, or proton beam irradiation. Although HSPs are upregulated to prevent cell apoptosis in times of stress, our findings may be explained by the fact that this upregulation is limited in time. Samali and Cotter<sup>37</sup> demonstrated in a fibrosarcoma and a monoblastoid cell line, that the cellular levels of HSPs reach a maximum after 6 to 8 hours of recovery after a heat shock. After 10 hours, there is a steady decline until the HSP levels return to normal at 20 to 24 hours. Because there is a minimum of a week between treatment of a uveal melanoma and enucleation, this may support the notion that no improved expression of HSP was found in the treated group compared with the nontreated group.

In recent years, there has been a growing interest in immunotherapy for tumors, including uveal melanoma. It has been stated for many decades that immunization against cancer is possible and that this immunization can be made specific for each individual.<sup>38</sup> In recent experiments in animal models, it has been possible to reduce tumor growth and even induce resolution of the primary uveal melanoma, mainly by the use of cytotoxic T cells (CTLs).<sup>39-42</sup>

If the problem of the low immune reaction against uveal melanoma lies in an inadequate antigen presentation to the CTLs, it may be possible to restore the immune reaction



by injecting mixtures of tumor peptides and HSPs. Because different antigen-presenting cells have a specific receptor for GP96 and other HSPs, this could establish a quick and effective activation of the CTLs, as experiments in mice have shown.<sup>43, 44</sup> In other tumors such as colorectal and renal cell carcinoma,<sup>16</sup> it has been stated that these HSP mixtures can provide a preventive or therapeutic tumor-specific immunity.

Recently, the first results of an HSP vaccination for cutaneous melanoma were announced.<sup>16</sup> After five injections, 50% of the patients showed activation of antimelanoma specific T cells. Clinically, of 28 patients, two had durable complete responses and three had long-term stabilization with vaccination based on GP96 peptide complexes.

One of the major problems is the need for at least 1.5 to 2 g of tumor tissue to be able to extract enough vaccination material. In our experience, large uveal melanomas weigh about 1.5 g, which could be enough for this type of vaccination. Because eyes with large uveal melanomas are mostly enucleated and usually lead to a high incidence of late metastasis,<sup>1</sup> this tumor is suitable for antimetastasis vaccination. An advantage of HSP-mediated vaccination is that specific tumor antigens do not have to be specified. The cancer-derived HSP-peptide complexes would elicit immunity to all the mutated epitopes of the malignancy. In conclusion, a precondition for the HSP vaccine approach is abundant expression of HSP70 or GP96 in the tumor tissue. Our results indicate that GP96 in uveal melanoma could be used as a vector for tumor vaccination in patients with large uveal melanomas.

## CONCLUSION

In uveal melanoma, HSP expression did not correlate to known prognostic significant factors. No differences in expression of HSPs were found between pre-enucleation-treated tumors and those not treated before enucleation, suggesting that there is no higher immunogenicity of the tumor after thermotherapy or radiotherapy due to HSPs. A low expression of the heat-inducible HSP70 was found in 15 of 20 uveal melanoma tumors without prior treatment. In contrast, GP96 was stably and homogeneously expressed in most uveal melanomas without prior treatment. Therefore, GP96 peptide complexes have potential for vaccination in large uveal melanomas.

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