

Review

Clinical, Histopathological and Cytogenetic Prognosticators in Uveal Melanoma – A Comprehensive Review

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Abstract. Uveal melanoma is the most prevalent primary intraocular cancer in adults. Although it accounts for only 5% of all melanomas, it is responsible for 13% of deaths due to this type of cancer. A wide variety of therapeutic options of primary tumor is available and progress in its management is noticeable. The fact still remains, however, that almost half of patients develop metastases which may be due to practically undetectable cancer spread present as early as at diagnosis of the primary focus. Metastatic disease is uniformly fatal despite systemic therapy. Prediction of metastasis is crucial for prognosis. It also allows targeting of emerging new therapeutic methods to the appropriate group of patients. The Authors reviewed literature concerning epidemiology and etiopathogenesis of uveal melanoma, and its clinical, histopathological and cytogenetic prognosticators.

Uveal melanoma (UM) is a potentially lethal cancer but factors determining unfavorable course of this disease are still unknown. Severe form of UM requires intense therapy. On the other hand, unreasonable treatment affects negatively the quality of vision and whole life of the patients. The decision on the scope of treatment needs to precede uniformly fatal metastatic spread. Consideration of clinical, histopathological and cytogenetical prognosticators help to define group of patients with different prognosis in UM.

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Epidemiology and Etiopathogenesis

Uveal melanoma (UM) is the most prevalent type of primary intraocular neoplasm in adults (1). In the European population, the incidence of UM ranges from under 2 to over 8 per million annually and is similar to the mean value of 4.3 per million for the USA. No significant changes of UM prevalence have been observed in either of the populations in long-term clinical observations (1-4). The incidence rate increases with age and tends to plateau among adults 75 years and older. The mean age at UM diagnosis is 58-61.4 years (± 15 years; range 3-100 years) and has increased gradually over the past 40 years (5, 6). Studies report slightly a higher incidence rate among men (2, 3, 5).

Most cases of UM occur in the White population (95-98%) (3, 5-7). In the European population, a latitudinal gradient of incidence was reported: in the south of Europe, the rate was lowest and increased towards the north to reach the highest values in Scotland and Scandinavian countries (2). In the USA, no geographical variations were noted except for in Hawaii where the incidence rate was considerably lower (one per million) (3). The authors of both reports pointed to the protective role of increased skin pigmentation and darker eye color as the cause of the observed correlations.

Despite progress in the management of primary tumor, the mortality rate for UM has remained high (1, 8). Although UM accounts for only 5% of all melanomas, it is responsible for 13% of deaths due to this type of tumor (4, 9). A wide variety of therapeutic options, namely brachytherapy, proton beam irradiation, transpupillary thermotherapy, photocoagulation, local resection, endoresection, and enucleation, is available. The fact still remains, however, that almost half of patients with UM develop metastases, which may be due to practically undetectable neoplasm spread present as early as

at diagnosis of the primary focus (10). UM spreads through the blood, and the liver is the preferred metastatic site (89%), followed by the lungs (29%) and bones (17%) (11). Uveal melanoma-related mortality reaches 31% by 5 years from the diagnosis of UM, and 45%, 49% and 52% by 15, 25 and 35 years, respectively (12). Therefore, it seems that the oncological follow-up should span at least 15 years after completion of primary tumor treatment.

It is common practice that all patients with UM are screened for liver metastasis every 6 or 12 months using imaging techniques or liver function tests, even though adjuvant therapy fails to yield satisfactory therapeutic effect and metastasis can hardly ever be managed by surgery (13-16), liver chemoembolization (17-19) or immunotherapy (20).

While the 5-year relative survival among patients with UM ranges from 77% to 84% (21), the mean survival time following detection of UM metastasis is 3-4 months, with subsequent 1-year survival of 10-15%, and only 1% of patients live longer than 5 years after diagnosis (11, 22). In patients who developed primary metastasis to sites other than the liver, the mean survival time is normally longer and is 19-28 months with statistical 1-year survival of 76% (23-25).

Etiopathogenesis of UM remains unclear. Guanine nucleotide-binding protein subunit alpha-Q (*GNAQ*) and guanine nucleotide-binding protein subunit alpha-11 (*GNA11*) gene mutations have been shown to be the first element in the chain of changes that lead to the development of most cases of UM (26, 27), and to be related with the activation of mitogen-activated protein kinase (*MAPK*) pathway and other intracellular signaling pathways (28, 29). It was demonstrated that the co-existence of *GNAQ* mutation and breast cancer 1-associated protein 1 (*BAP1*) gene mutation is associated with increased metastatic potential of UM. Families with *BAP1* germline mutations are more susceptible to a number of cancer types including choroidal melanoma (30-32), however, *BAP1* mutations are usually somatic, and the prevalence of hereditary UM is lower than 1% (33). The role of environmental factors in UM pathogenesis is still elusive, although sunlight exposure has been implicated in development of *GNAQ/GNA11* mutation (34-36).

Clinical Prognosticators

The clinical and histopathological features that are predictive of poor prognosis of UM are: older age, ciliary body location, large basal diameter and thickness of tumor, the presence of closed connective tissue loops, epithelioid cell type, high mitotic rate, extraocular tumor extension, brown color of the tumor, presence of subretinal fluid and intraocular hemorrhage (6, 37, 38).

i. Patient age at diagnosis. The work by Shields *et al.*, which investigated 8,033 cases, is the largest study available to date

discussing the prognostic significance of age in UM (39). It was shown that 1% of UM is diagnosed in patients below 20 years of age, 53% those aged 21-60 years and 45% in patients over 60 years old. Young patients are relatively often diagnosed with tumors of the iris (21%), while in all age groups, UM is most commonly located in the posterior part of the uvea (71, 91 and 90%, respectively) (39).

Mean basal tumor diameter and thickness, as well as the frequency of extrascleral extension, were shown to increase with age, similarly to the rate of occurrence of metastases and tumor-related mortality (39).

Metastasis at 3, 5, 10 and 20 years was detected in patients under 20 years old in 1.7%, 8.8%, 8.8% and 20.2% of cases, respectively, in the group aged 21-60 years in 6.2%, 12.2%, 23.0% and 34.2% of cases, and in those over 60 years old in 11.1%, 18.7%, 27.7% and 38.8% of cases (39).

The corresponding 3-, 5-, 10- and 20-year tumor-related mortality rates were 0%, 2.2%, 5.1% and 17.0%, 3.2%, 6.2%, 11.0% and 16.6%, and 6.5%, 11.0%, 15.9% and 20.1%, respectively (39).

ii. Tumor location. Although UM may be potentially diagnosed on clinical examination with almost 100% accuracy (34), in many patients, the tumor is missed or misdiagnosed (40). A lack of subjective symptoms in nearly one-third of patients, comorbid eye disorders, or diseases which hinder diagnosis, as well as incorrect technique (*e.g.* examination without mydriasis) may be the root of some of the problems. Tumor location may also make diagnosis more difficult: 3.5% of UM occurs in the iris, 6.1% in the ciliary body, and 90.3% in the posterior part of the uvea (6).

UM located in the ciliary body initially does not result in visual impairment and often remains undiagnosed until it is large enough to distort the iris or emerge from beneath its edge and induce other structural and functional changes within the eyeball. Sometimes it is diagnosed only after extraocular spread is observed. Even though ciliary body location of UM is an unfavorable prognostic factor irrespective of its size or cellular type (41), it was demonstrated that UM involving the ciliary body is more likely to comprise epithelioid cells and have a greater diameter than that confined to the posterior part of the uvea (42). It is assumed that an anatomical location that impedes early diagnosis, as well as high mobility of the ciliary body due to the contractions of the ciliary muscle, the numerous vessels and a likely occurrence of extravascular matrix patterns of poor prognosis in this area may be associated with higher metastatic potential of UM (42, 43). Delayed treatment often necessitates a more radical form of treatment *i.e.* enucleation of the eye (40).

UM that originates from the iris is associated with better prognosis, which may stem from the fact that such tumors are more easily identified and, as a result, treatment is applied

much sooner (41). Clinical features that affect the metastatic potential of UM of the iris are age at diagnosis, infiltration of the anterior chamber angle, extraocular extension, elevated intraocular pressure and prior surgeries (44).

Tumors of the posterior part of the uvea, which directly or indirectly (*e.g.* due to concomitant retinal detachment) affect the macula, may induce blurry and distorted vision. It seems that these symptoms should contribute to cancer being diagnosed earlier than in the case of ciliary location. However, in the study by Shields *et al.*, a relatively small difference was reported as regards patient age at diagnosis (6). For UM of the iris, ciliary body and posterior part of the uvea, it was reported as 50, 59 and 58 years, respectively.

At diagnosis, the mean thickness of tumor located in the iris was 2.7 mm, 6.6 mm in the ciliary body, 5.5 mm in the posterior part of the uvea, and the mean largest basal diameter was 6.5 mm, 11.7 mm, and 11.3 mm, respectively. At 3 years, metastases were observed in 0.5%, 12%, 8% of cases, respectively, at 5 years in 4.1%, 19%, 15%, and at 10 years in 6.9%, 33%, 25% (6).

5-Year mortality for UM of the ciliary body was reported to be 22-53% and for that of the posterior part of the uvea 14%, and 10-year mortality for iris UM was 5-6% (41).

iii. Tumor size. Meta-analysis of Diener-West *et al.* attempted to provide systematic results of eight studies on mortality rates following enucleation for UM (45). For small (<3 mm-thick and <10 mm in basal diameter), medium (3 to 8 mm-thick and <15 mm in basal diameter) and large (>8 mm-thick and >15 mm in basal diameter) tumors, 5-year overall mortality was 16%, 32% and 53%, respectively.

Shields *et al.* adopted tumor thickness as the criterion of tumor size, as they decided that the acquisition of this dimension by ultrasonography ensures higher precision than the measurement of basal tumor diameter (6). They concluded that small tumors (0 to 3.0 mm-thick) metastasize at 5, 10 and 20 years in 6%, 12% and 20% of cases respectively, medium (3.1 to 8.0 mm-thick) tumors in 14%, 26% and 37%, and large (>8.0 mm-thick) tumors in 35%, 49% and 67%. Every 1 mm increment in thickness of primary UM equated to a 5% higher probability of metastasis development and *e.g.* for a 4 to 5 mm-thick tumor, the probability was approximately 25%, while for one 7 to 8 mm-thick, it increased to 40% (6).

In another study, Shields *et al.* compared the prognosis for small tumors (<3 mm-thick) of diffuse (thickness/base ratio $\leq 20\%$) and nondiffuse (thickness/base ratio $>20\%$) types (46); 17% of tumors were diffuse. UM-related metastasis was detected in 8% and 4% of cases, respectively, at 5 years, 16% and 10% at 10 years, and 19% and 16% at 15 years. The corresponding UM-related mortality rates were 6% and 2%, 11% and 4%, and 16% and 6%, respectively. This considerably worse prognosis for patients with diffuse

tumors was even more prominent when tumors up to 2 mm-thick were analyzed.

Tumor basal diameter and its thickness have been reported to be strongly correlated with UM prognosis by numerous authors (38, 47, 48).

iv. Methods of treatment. The aim of the multicenter Collaborative Ocular Melanoma Study (COMS) was to solve the problem of selecting the optimum method of treatment for primary UM (34). The three-arm study evaluated the effectiveness of management of small, medium and large tumors (1.5 to 2.4 mm-thick and 5-16 mm in diameter, 2.5 to 10 mm-thick and ≤ 16 mm in diameter, >10 mm-thick and >16 mm in diameter, respectively).

In the case of large tumors (1,003 patients), the effectiveness of enucleation was compared to enucleation with preoperative radiation therapy. 5-Year survival was not statistically different between the groups and 5-year tumor-related mortality was 28% and 26%, respectively (34).

For medium tumors (1,317 patients), enucleation was compared with brachytherapy using iodine-125. The mortality rate in both groups was almost identical. It should be noted, however, that ciliary body location tumors and tumors located near the optic nerve disc which are inaccessible for brachytherapy were excluded from the COMS (34).

A group of 204 patients diagnosed with a small UM was entered into a registry and managed with watchful waiting. 5-Year and 8-year cancer-related death was 1% and 3.7%, respectively (34).

Even though COMS received criticism due to the study criteria applied, the data collected brought about a shift in treatment modalities for small and medium tumors towards vision-preserving therapies.

Similar survival times of patients with primary UM treated by different methods (34, 41) prove that improving diagnosis and management of metastasis is vital for prognosis.

Histopathological Prognostic Factors

i. Cell type. The first classification of malignant melanocytic eye tumors was proposed in 1931 by Callender (49), and then modified by McLean *et al.* (50).

The main criterion of division into histologic types of UM is the morphological subtype of cells as epithelioid or spindle cell in the tumor. Epithelioid cells have abundant acidophilic cytoplasm, large round or oval nuclei, a high nuclear-to-cytoplasmic ratio and a high number of mitotic figures. They are large, polymorphous and have a tendency towards discohesion. Their presence in the tumor is strictly related with considerably higher UM metastasis development probability and higher mortality rate (38, 51).

Spindle cells are elongated with large nuclei and scant cytoplasm (low nuclear -to-cytoplasmic ratio). They are

uniformly and densely arranged and may form palisades. There are very few cells with prominent nucleoli, and hardly any mitotic figures are observed.

The epithelioid cell type comprises approximately 3-5% of all UM and is associated with the least favorable prognosis. The 15-year mortality rate among patients diagnosed with epithelioid cell type UM is 75% (41).

Spindle cell type accounts for approximately 40% of all UM. The 15-year mortality rate is 20% (41).

Up to 50% of all UM are the most frequent mixed type. The 15-year mortality rate is approximately 60% but considerable differences are observed depending on the percentage of epithelioid and spindle cells (41).

ii. Mitotic activity. McLean *et al.* demonstrated a strong association between the number of mitoses observed per 40 high-power fields (HPF) and prognosis in patients with UM (51). Tumors of low mitotic activity (0-1/40 HPF) were associated with 6-year mortality rate of 15-23%, those with medium activity (2-8/40 HPF) 40-47%, and those with high activity (9-48/40 HPF) 56%. In a study by Damato *et al.*, there was a statistically significant correlation between tumors with mitotic activity higher than 4/40 HPF and the development of metastasis and metastasis-related mortality (38). Angi *et al.* demonstrated that the conventional mitotic activity count that involves counting mitotic figures in a routine hematoxylin and eosin staining depends on the experience of the person performing the count and carries an error of underestimation (52). They demonstrated that the use of phospho-histone H3 (Ser10) mitotic marker results in a higher number of mitoses being recognized in the analyzed material, increasing the reproducibility of counting and makes it more independent of the examiner's experience.

iii. Tumor-infiltrating lymphocytes (TILs) - lymphocytic inflammatory infiltration. Globally, inflammatory infiltration in UM, which involves an increased number of lymphocytes and macrophages and human leukocyte antigen (HLA) I and HLA II expression, is associated with worse prognosis (53).

Lymphocytic infiltration is observed in 17% of cases of UM (54), more frequently in ciliary body UM than in tumors confined to the posterior part of the uvea (42). TILs in UM are mainly suppressor/cytotoxic T-lymphocytes (CD8⁺), to a lesser degree T-helper lymphocytes (CD4⁺), as well as regulatory T-lymphocytes [CD3⁺ forkhead box P3 (FOXP3)⁺] (55, 56).

Lang *et al.* showed that brisk and non-brisk lymphocytic infiltration was associated with mixed and epithelioid cell UM (93.3% and 78.4%, respectively) (54). However, no statistically significant difference in mortality was demonstrated in their study in relation to TILs presence or absence in UM. In a study by de la Cruz *et al.* brisk TILs (defined as the presence of 100 or more lymphocytes per 20 HPF) was observed in 12.4% of

UM (57). A group of 125 tumors with brisk TILs was compared with a control group with less intense inflammatory infiltration and it was shown that TILs are a significant factor of adverse prognosis in patients with UM. The 15-year survival rate in the group of patients with low lymphocytic infiltration is up to 69.6% and falls to 36.7% in the group with high infiltration. The authors suggested that the presence of TILs results from the general response of the immunological system to melanoma cells in the circulatory system, which act as specific circulating tumor cells (CTCs) that are a potential source of metastasis. In this approach, TILs identified in the primary UM should be considered indirect evidence of systemic cancer spread (57).

Better prognosis observed with down-regulation of HLA class I on UM cells is explained by their higher susceptibility to natural killer lymphocytes (53).

The influence of T-regulatory lymphocytes on the course of UM and prognosis remains unclear. There are some hypotheses that they may be involved in the induction of immunosuppressive mechanisms that block the proper response to the tumor (53).

Tumor-associated macrophages (TAMs) of varying grades also occur in UM with infiltration being low in 17%, moderate in 51%, and pronounced in 32% (58). The percentage of macrophages in lymphocytic infiltration is correlated with metastasis-related mortality and female sex, largest basal diameter of the tumor, epithelioid cell type, strong pigmentation and microvascular density (58). Most of the TAMs that are found in UM belong to the M2 subgroup which supports tissue remodeling and angiogenesis (59).

iv. Extravascular matrix pattern. Folberg *et al.* described nine morphological patterns of UM extravascular matrix (60). They found that the patterns are created by the tumor vessels, which gave rise to controversies (61, 62). Irrespective of the ongoing research into the origin of the connective tissue septal networks (containing blood vessels) between the complexes of tumor cells observed in periodic acid-Schiff staining, Folberg *et al.* demonstrated that their arrangement affects prognosis (60). They described two patterns, namely loops, and networks, whose common feature is the presence of at least one closed loop. Then they compared the patterns that did not contain a closed loop with the ones that did. During the follow-up, UM ended in the death of two patients in the first group (19 tumors) and of 18 patients in the second group (21 tumors), respectively. Mäkitie *et al.* demonstrated that the extravascular matrix pattern may be determined in 80% of UM cases acquired as a result of enucleation (63). The loop pattern was identified in 60% of cases and the network pattern in 35%. Ten-year UM-related survival was statistically worse for those with network pattern tumors, compared with those loops without networks and those with no loops (0.41 *versus* 0.53 *versus* 0.83; $p < 0.0001$), and the prognosis for tumors

with a network pattern and those with loops without networks did not differ significantly.

Rummelt *et al.* stated the strong association of ciliary body UM with death from metastatic melanoma ($p=0.0006$), along with the following extravascular matrix patterns: parallel vessels ($p=0.0043$), parallel with cross-linking ($p=0.0001$), arcs ($p=0.0028$), arcs with branching, loops and network ($p=0.0001$ each). The aggressive behavior of ciliary body melanomas appears to be related to the tendency for vascular networks to develop in this location. Regardless of location, ciliary body or choroid, the presence of vascular networks shortens survival (42).

Connective tissue septa that form extravascular matrix patterns associated with worse prognosis were found to contain a higher number of blood vessels (62). This means non-invasive methods of assessment vascularization for UM prognosis might be applied (64, 65). Characteristic extravascular matrix patterns are also reported in UM metastases, irrespective of their location (66). Further studies concerning the origin and role of tissues that form extravascular matrix patterns may help improve UM therapy.

v. Degree of pigmentation. McLean *et al.* showed that the degree of tumor pigmentation has no prognostic value for patients with tumors containing epithelioid cells (51). On the other hand, prognostic value assigned to the amount of pigment in spindle-cell tumors may to a large extent be attributed to artifacts produced in the course of preparation of histopathological specimen. Accurate definition of the prognostic significance of pigmentation warrants further histopathological and statistical analysis.

vi. Extrascleral extension. Extrascleral extension may occur *via* aqueous channels (29.8%), ciliary arteries (27.4%), vortex veins (18.5%), ciliary nerves (8.9%) or the optic nerve (0.8%). In 10.4% of patients, the tumor spreads to the extraocular space simultaneously *via* a variety of routes.

Extrascleral extension is strongly correlated with involvement of the anterior chamber angle, basal diameter of the tumor, the presence of epithelioid cells, closed connective tissue loops and monosomy 3. Interestingly, extraocular extension along aqueous drainage channels, which is the most frequent route, correlated positively with anterior chamber angle involvement and inversely with basal tumor diameter and tumor thickness.

Metastasis-related mortality is higher irrespective of the route of extrascleral spread route and was not found to correlate with the size of extraocular tumor. It seems that extrascleral tumor extension may be considered incidental to tumor malignancy, and in the case of UM of the posterior part of the uvea, also to its stage of advancement rather than being an independent cause of development of distant metastases (37).

In a study by Schmittel *et al.*, 5-year metastasis-free survival in patients with extraocular extension of UM was 28% *versus* 80.6% among patients without extraocular tumor growth (48). The mean time to metastasis development in patients with extraocular tumor growth was 35 months. The 5-year survival was 50% among patients with extraocular tumor growth and 83.3% among those without.

Cytogenetic abnormalities as potential prognosticators

The correlation between UM and changes within chromosomes 1, 3, 6 and 8 was noted a long time ago. The most frequent aberrations include the loss in the short arm of chromosome 1 (27%); losses in the short arm (45%), and long arm (49%) of chromosome 3; loss in the long arm (39%), and gain in the short arm (39%) of chromosome 6; loss in the short arm (20%), and gain in the long arm 8 (69%) and the short arm (20%) of chromosome 8 (67-69).

Hoglund *et al.* confirmed the existence of two independent cytogenetic pathways in UM that are of key importance for UM progression (68). The early alteration of one of them is the occurrence of monosomy 3, and subsequent 8q+, 8p- and 1p- aberrations. In the second one, gain of the short arm of chromosome 6 and subsequent 6q-, 8q+ imbalances occur. Co-occurrence of monosomy 3 and 6p imbalance is reported in only 4% of UM, while in 18% of primary tumors, the proper number of chromosomes 3 and 6p was observed (69). Furthermore, similar cytogenetic aberrations were observed in cutaneous melanoma. This is intriguing considering the many discrepancies identified clinically in the course of melanoma in both locations (68). Taking into account clinical data, it was shown that the subgroup of patients with monosomy 3 and normal short arm of chromosome 6 was associated with the highest probability of development of metastatic foci. Indirect risk was identified for the subgroup with disomy 3 and 6p+, and the lowest for the subgroup without aberrations in 3 and 6p (69).

Monosomy 3 is detected in approximately 50% of primary UM. It is an independent unfavorable prognostic factor of shorter survival and a factor of higher risk of developing organ metastasis (more prominent than any other clinical factor) (70). In a study by Prescher *et al.*, during the follow-up period UM metastasis was detected in 57% patients with confirmed monosomy 3 in the primary tumor (70). The mean survival in this subgroup was less than 6 months from the diagnosis of metastasis. The authors also suggested concomitant involvement of several genes located on chromosome 3 in the changes occurring in the course of UM progression and the secondary development of distant metastases. Suppressor genes, whose inactivation conditions the development of UM, may be located both on 3p and 3q, which might explain a correlation with the loss of the entire

(normal) chromosome (70, 71) that is more frequent here than in other cancer types.

The selection of examination technique is an important issue in the search for monosomy 3. Isodysomy 3 may occur in the UM progression as a result of the loss of one chromosome 3, and the subsequent duplication of the faulty chromosome (72). Some of the methods of karyotype evaluation only allow detection of "standard" monosomy, which leads to the underestimation of the number of faulty cells. Techniques based on the analysis of the number of chromosomes, such as fluorescence *in situ* hybridization and comparative genomic hybridization, have lower sensitivity and specificity in the prognosis of UM metastasis than methods allowing for the detection of the loss of heterozygosity of chromosome 3, such as single nucleotide polymorphism (73, 74).

It must be stressed that although a strong correlation between monosomy 3 and prognosis in UM was confirmed, this factor cannot be the only prognostic criterion. Clinical assessment is of key importance for the precise prognostic stratification of patients with UM, with special attention paid to the basal tumor diameter and identification of the histopathological type of UM. Genetic typing should also allow for other mutations and aberrations specific for UM (38, 74).

8q+ Chromosomal aberration is the one most frequently observed in UM. Therefore, the order of occurrence of monosomy 3 and 8q+ in the course of UM, as well as the role of either of these as the tumor-initiating aberration, are still controversial (69, 75). The number of copies of the long arm of chromosome 8 may increase as a result of its gain (8q+/8pnl) and as a result of trisomy 8 (8q+/8p+) or isochromosome 8 (8q+/8p-) that in UM occurs almost exclusively with monosomy 3 (69). Irrespective of the chronology of changes, we know that 8q+ is more frequently found in patients with monosomy 3, which worsens prognosis depending on the number of copies of the long arm of chromosome 8 (69, 75). Monosomy 3 and an increased number of chromosome 8 are strongly correlated with the involvement of ciliary body, basal tumor diameter, epithelioid cell UM, the presence of closed loops, and high mitotic rate. Furthermore, monosomy of chromosome 3 is strictly correlated with the presence of extraocular spread (38). Damato *et al.* determined that 5-year UM-related mortality was 6% with no aberrations in chromosomes 3 or 8, 31% with gain of chromosome 8, 40% with monosomy 3, and 66% with co-existent monosomy 3 and chromosome 8 gain (38). However, the study was only focused on tumors originating in the posterior part of the uvea.

In UM, changes occur in the genes that are related with centrosomal function, regulation of the cell cycle and DNA damage repair *i.e.* the key processes in maintenance of genomic integrity. Ataxia telangiectasia and Rad3 related (*ATR*) and phosphatase and tensin homolog (*PTEN*) gene

expression is decreased in UM. Mutations of *ATR* gene located on chromosome 3q22 are responsible for microsatellite instability. Mutations of *PTEN* tumor-suppressor gene lead to the loss of genomic integrity *via* a number of ways. The occurrence of *ATR* and *PTEN* gene damage may partly explain the gradual increase of aneuploidy in melanoma development (69). However, Ehlers *et al.* showed that poor prognosis in UM is related with early chromosomal alterations rather than with the subsequent increase in their number (69).

The use of gene-expression profiling by Onken *et al.* (76) allowed for identification of 62 discriminating genes in UM. They were used to divide the analyzed tumors into two classes. Class 2 tumors had a lower number of genes with their loci on chromosome 3 and a higher representation of genes of the long arm of chromosome 8, which was in agreement with previous studies on chromosomal aberrations in UM. To determine the prognostic value of the classifier in UM metastasis, 50 cases were analyzed. The 92-month survival probability was 95% in class 1 and 31% in class 2. The prognostic accuracy of molecular classifier superseded all clinical and histopathological prognostic factors. It should be noted that half of the tumors were evaluated based on the sample that corresponded to the size of the material acquired at biopsy (76). Class 1 was further divided into subclass 1a and 1b that differed as regards prognosis, with 5-year cancer-related mortality of 2% and 21% for class 1a and 1b, respectively, and 72% for class 2 (77). Class 2, associated with a high risk of developing metastasis, was found to be correlated with frequent occurrence of *BAP1* gene mutation at the 3p21.3 region. Harbour *et al.* identified *BAP1* mutations in 26 out of 31 (84%) class 2 UM and in only one out of 26 class 1 UM (78). Class 2 was associated with the largest basal tumor diameter, poor cellular differentiation and epithelioid cell tumor, involvement of ciliary body, the presence of extraocular spread, and closed loops, as well as the loss of heterozygosity of chromosome 3, increased aneuploidy, higher mitotic rate and up-regulation of Ki-67 (33). Continuing their studies into molecular classifier of UM based on gene expression profiling, Onken *et al.* proposed that the number of analyzed genes be reduced to 12 discriminating genes and three control genes (79). The use of polymerase chain reaction has made the evaluation largely independent from the method of obtaining the material for testing. Additionally, the authors showed that heterogeneity of tumor tissue does not have a significant impact on the result of tests performed on the material obtained using fine-needle aspiration biopsy. Modifications of this method help to optimize it for routine clinical applications and for eye- and vision-sparing therapies.

Estimating prognosis in clinical practice, particularly in individual patients, requires prior validation of research methods (80). The Collaborative Ocular Oncology Group, which analyzed results from 12 independent research centers,

showed in its first report that gene-expression profiling supersedes the evaluation of chromosome 3 and TNM classification to offer more accurate prognosis as regards the metastatic potential of UM (81). Gene-expression profiling allowed UM molecular class to be determined in 97.2% of cases, of which 61.9% were class 1 and 38.1% class 2. Throughout the follow-up period (18 months on average), UM metastases were detected in 1.1% of cases in class 1 and 25.9% in class 2 (81). This allowed a commercially available test to be introduced into the market (DecisionDx-UM; Castle Biosciences, Incorporated). It is now used in several centers (82, 83).

The excellent progress made over the past decade in molecular biology of UM will enable for further development of new prognostic tests that will stratify patients into prognostic subgroups that differ significantly as regards long-term prognosis and risk of distant metastases with even higher precision and accuracy. Moreover, advances in molecular biology may help discover new signaling pathways involved in UM development, which may pave the way towards new target drugs for personalized therapy.

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