
Molecular Carcinogenesis of Uveal Melanoma

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Abstract

Melanocytes are derived from the neural crest and enter the eye during embryogenesis. Uveal melanoma of the eye is a rare but deadly disease. About 50% of patients will eventually develop metastatic disease with an inevitable fatal end. Predisposing factors are race, skin and hair color, and familial tumor predisposition syndromes.

Although clinically uveal melanoma phenotype gives the impression of one disease, genetically uveal melanoma can be classified into at least two subgroups which can be distinguished by DNA-based and mRNA-based technologies. While patients with disomy 3 and gene expression profile class 1 have only a low risk of developing metastatic disease, patients with monosomy 3 and/or class 2 gene expression profile are likely to die from metastases. In addition to prognostic information, genetic testing also provides new insights into molecular pathology of uveal melanoma. Mutations in *GNAQ*, *GNA11*, and *BAP1* have been found to be the crucial steps in tumor development.

Those insights raise the hope for targeted therapies and improved prognoses for uveal melanoma patients in the near future.

9.1 Background

Melanocytes are found ubiquitously in the eye and periocular region. It is our current understanding that during embryogenesis pigment cells derived from the neural crest migrate along the nerve sheaths of the trigeminal branch V1 and reach the eye via the branches of the ciliary nerves. Still being melanoblasts, those cells enter the eye close to the optic disc to be distributed throughout the uvea—the choroid, the ciliary body, and the iris—where they mature and become melanocytes [1]. Disturbance of this process of maturation may lead to proliferation of melanocytic cells. They appear benign, round, or oval in shape if the proliferation started after the cells had reached their final destination. The lesion may affect a large sector of the iris and choroid or even the periocular skin if proliferation began early in posteroanterior migration, at a central branch of the trigeminal nerve (e.g., nevus of Ota) [2, 3]. Additional genetic alterations acquired later in life may cause further growth and increased proliferation rate and finally result in the development of a malignant uveal melanoma. Over the past decade, our understanding of the molecular and genetic mechanisms which deregulate the cell cycle of melanocytes and eventually turn nevi into melanomas has increased significantly. Many researchers and research groups contribute to elucidation of the pathogenesis of uveal melanoma, with the ultimate goal of finding a cure for or even prevent this disease.

In the following chapter, we present the current status of research in the molecular carcinogenesis of uveal melanoma, show how the clinical appearance results from molecular and genetic mechanisms, and demonstrate the clinical impact which might result from those. For readers who are not experts in ophthalmic oncology, we will give a brief general overview of the disease, as far as this is necessary for the understanding.

9.2 Epidemiology of Uveal Melanoma

The incidence of uveal melanoma varies among different races and ethnicities over the world. An analysis of the SEER (Surveillance, Epidemiology, and End Results) program database in the United States covering 36 years, from 1973 to 2008, revealed a mean age-adjusted incidence of 5.1 cases per million per year [4]. However, the majority of cases (97.8%) were observed in the white population. The ratio of black population-Asian population-Hispanic population-non-Hispanic white population is 1:1.2:5:19 (SEER program data, 1992–2000) [5]. Whereas the incidence rate was not significantly different between the registries in the United States, an analysis of the data from the European Cancer Registry-based (EUROCARE) study, which combined the data from 67 individual European cancer registries over the years 1983–1994, showed a dependency on latitude. The incidence decreased from north to south, with eight cases per million per year in Scandinavia and only two cases in the south of Europe (Spain and Southern Italy) [6, 7]. In comparison to cutaneous melanoma, with an incidence of 21.8 cases per 100,000 men and women per year, uveal melanoma appears rare (data from SEER Stat Fact Sheets 2016). Only 5% of all melanomas arise from the eye [8]. However, uveal melanoma is still the most common primary intraocular malignancy in adults [9].

9.3 Predisposing Factors

Predisposing factors can help elucidate the genetic mechanisms underlying carcinogenesis. Epidemiological, familial, clinical, and occupational factors contribute to tumor development. The incidence of uveal melanoma increases up to an age of 70 years, and the incidence peaks at 24.5 cases per million males and 17.8 cases per million females per year in the United States, resulting in a mean age at diagnosis of 60 years [6]. Overall, sex does not seem to be a predisposing factor. Data from the EUROCARE study and from the United States demonstrate that race (see the above), skin and eye color, and the ability to tan are the main predisposing factors [4, 7, 10]. This suggests a possible role of UV radiation in the carcinogenesis of uveal melanoma [11–13]. Though this hypothesis is still under discussion and there is no good evidence available, the preferred occurrence of iris melanoma in the inferior half of the iris and the higher incidence of choroidal melanomas at the posterior pole of the eye could be explained by a higher exposure to UV light in those locations. A cohort effect found in the data from the EUROCARE study, with a higher incidence of uveal melanoma in the birth cohorts from 1910 to 1935, might be interpreted that way, as changes in the profiles of work and occupational UV light exposure and the use of sunglasses could explain the decrease in incidence in the younger cohorts [7].

Familial uveal melanoma: Occurrence of uveal melanoma in more than one family member is a rare event, accounting for only 0.6% of patients [14]. However, several cases have been reported, dating back until 1905 [15]. In the published cases, no pattern of inheritance could be found, and only few individuals were

affected in each family [14–16]. Other features of a genetic predisposition, such as early age at diagnosis, bilateral involvement, or phenotypic associations were not present [17]. Therefore, it might be possible that two individual family members might be affected by chance alone, though the likelihood of such an occurrence is small [18]. On the other hand, uveal melanoma has been reported to occur more frequently in patients showing oculodermal melanocytosis, familial atypical mole and melanoma syndrome, neurofibromatosis, Li-Fraumeni syndrome, or germline BAP1 mutations, which all have a hereditary and therefore familial background.

Oculodermal melanocytosis: Patients with oculodermal melanocytosis present with congenital increased pigmentation within the distribution of the first and/or second branch of the trigeminal nerve. The affected organs include not only the periocular skin/eyelid, episclera/sclera, uvea, and orbit but also the meninges and tympanic membrane. Patients with oculodermal melanocytosis have a significantly increased risk for uveal melanoma, with an estimated lifetime risk of 1:400 for developing uveal melanoma [19]. It remained unclear whether the increased risk for uveal melanoma results simply from the increased number of melanocytes in the uvea or from an underlying predisposing condition. The large affected sector of the trigeminal nerve points toward an early event in melanogenesis, altering melanoblasts before final maturation [3]. The frequent occurrence of mutations in GNAQ found in intradermal melanocytic proliferations/blue nevi and uveal melanomas appears to drive melanocytic proliferation and might explain the increased risk of malignant transformation [20].

Familial atypical mole and melanoma syndrome (FAM-M): According to the NIH consensus conference, the FAM-M syndrome is diagnosed in individuals with multiple atypical cutaneous nevi, showing distinct histological features and with cutaneous melanoma in one or more first- or second-degree relatives [21]. Dysplastic or atypical nevi of the skin may also occur isolated and are associated with an increased risk of cutaneous melanoma [22]. However, the FAM-M syndrome is a hereditary syndrome, caused by a mutation of the CDKN2A gene, coding for the INK4a and ARF proteins, which regulate the cell cycle and act as tumor suppressors. Germline mutations in CDKN2A are associated with an increased risk of cutaneous melanoma, glioblastoma, and pancreatic cancer [23, 24]. Several case series suggested a role for CDKN2A and the FAM-M syndrome in the development of uveal nevi and melanoma. Uveal nevi and uveal melanomas seem to occur more frequently in patients with FAM-M syndrome. And the FAM-M syndrome has been observed more frequently in patients with uveal melanoma [25–32]. Despite this clinical evidence, the connection between FAM-M is still not fully understood and unproven, as CDKN2A mutations do not seem to play a role in uveal melanoma development and/or progression [33–36].

Neurofibromatosis-1 is a frequent autosomal dominant inherited genetic disorder affecting about 1 in 3000 individuals [37]. Because of the high prevalence, repeated cases of uveal melanoma in NF 1 patients may be coincidental. A connection has been suggested because half of uveal melanomas show reduced expression of NF1 tumor suppressor gene causing an increased activity of Ras and of MAPK activation [38].

Li-Fraumeni syndrome is an autosomal dominant inherited cancer predisposition syndrome caused by a germline p53 mutation [39].

BAP1 mutation: Germline mutations of BAP1 cause a hereditary tumor predisposition syndrome, and affected individuals frequently develop uveal melanoma, lung adenocarcinoma, mesothelioma, and meningioma [40–42]. The spectrum and number of tumors vary between individuals and the affected kindreds, and several cases of familial uveal melanoma have been associated with BAP1 mutations [43, 44]. The reduced penetrance of the germline mutation, however, can impede the identification of those patients as familial cases. Somatic mutations of BAP1 have been identified in metastasizing uveal melanoma and are thought to be a critical step for development of an aggressive tumor phenotype [45]. In case of a germline BAP1 mutation, loss of the wild-type allele of BAP1 on the remaining allele on chromosome 3 will promote melanoma development. The role of BAP1 mutations and the presumed genetic mechanisms are described later in this chapter.

9.4 Prognosis

Uveal melanoma is generally reported to have an overall chance of 50% for spreading to the liver and cause metastatic disease, which is usually fatal within a few months. However, for counselling patients, this information is not very helpful. It has been shown that for most patients, the prognosis is actually much better or much worse and that we need to obtain an individualized risk, by combining clinical, histological, and genetic risk factors [46].

Clinical factors: Older age and male gender have been associated with reduced survival. However, there remains the possibility that those results are biased by a delay in diagnosis in older patients, a higher general mortality rate in older patients (competing risks), and prolonged survival of younger patients with metastatic disease compared to older ones [47–50].

Histopathological factors: Uveal melanoma is commonly classified on cytomorphology according to a classification scheme proposed by Callender in 1931 and modified by McLean in 1983 [51]. Cells are divided into fusiform spindle cells and the larger polygonal and pleomorphic epithelioid cells. Both cell types may be present in the same tumor, which is then classified as “mixed cell type.” Epithelioid cell type has been associated with a higher metastasis rate [52]. Problems with the classification lie in the significant inter- and intraobserver variability and the missing consensus on how many epithelioid cells must be present for a melanoma to be classified as “mixed” or “epithelioid” [53]. Cytological tumor heterogeneity here indicates an underlying genetic heterogeneity. It has been shown by several authors that epithelioid cell type and cytogenetic risk factors (monosomy 3) are correlated [54, 55]. As the presence of even a low number of cells with monosomy 3 already causes deterioration of prognosis, agreement of a cutoff level for a histopathological classification appears critical. Recently, after the mutations in the gene encoding BRCA1-associated protein 1 (BAP1) on chromosome 3p21.1 have been identified as critical factor for the development of metastatic tumor phenotype, it was shown that

depletion of BAP1 resulted in less differentiated spindle morphology, again drawing a connection between molecular changes and histopathological findings [56]. In addition to epithelioid cell type, the presence of specific extracellular matrix patterns (closed loops; networks) has been found to be associated with poor prognosis [57–59]. Those patterns are best depicted on PAS-stained sections and were initially thought to represent blood vessels but then identified as fluid-conducting channels lacking endothelial cells (vasculogenic mimicry). As cell type, they also correlate with other risk factors for metastasis, reflecting putative molecular changes in the melanoma cells [60]. In addition, increased microvascular density and the presence of tumor infiltrating macrophages and lymphocytes worsen prognosis [61–63]. Today, histopathological risk factors have lost much of their importance for counseling patients about their likely prognosis, as the identification of genetic risk factors has been proven to be superior predictors. However, they are still in use in multivariate mathematical models for prognostication as the inclusion of numerous predictors enables the model to correct for errors in even genetic results to some extent [46, 60].

Tumor parameters: Tumor dimensions, especially the largest tumor basal diameter (LBD), are strongly correlated with patient survival. While less than 5% of melanomas with a LBD less than 10 mm will be fatal within 5 years from diagnosis, 5-year mortality rises to over 50% in melanomas with a LBD over 15 mm [64, 65]. LBD remains an independent risk factor adding prognostic information even in addition to gene expression profiling (GEP) [66]. However, we do not know whether metastasis happens because the tumor has grown large and has had more opportunity to spread because it has been there for a longer time or whether large tumor size is simply an indicator of a greater growth rate [46]. Tumor height appears to be a less important parameter for patient survival. Tumor location, on the other hand, has long been recognized as an important predictor of metastasis. Ciliary body involvement is associated with reduced survival; however, it correlates with larger tumor size and it did not remain an independent risk factor for metastatic disease when information from GEP was available [53, 66, 67]. Melanomas of the iris, in contrast, have a favorable prognosis and a much lower mortality rate [68, 69]. At the time of diagnosis, iris melanomas are usually smaller than choroidal or ciliary body melanomas, and they show less aggressive histological features [53]. Cytogenetic changes with known unfavorable impact could be demonstrated in iris melanoma as well. Due to the small number of cases, however, the impact on survival has not been analyzed in detail [70].

9.5 Cytogenetics

Since the first report on cytogenetic changes in uveal melanoma more than 30 years ago, cytogenetic testing has gradually evolved from a research tool to a routine clinical test in the management of uveal melanoma [71]. Twenty years ago, Prescher et al. demonstrated the prognostic significance of cytogenetic abnormalities (i.e., monosomy 3), which was shown to be far superior to any clinical or histopathological marker [72]. Since then, the techniques to identify chromosomal anomalies have evolved and replaced chromosome G-banding. Today, fluorescence in situ

hybridization (FISH), spectral karyotyping (SKY), (array) comparative genomic hybridization (a-CGH), and multiplex ligation-dependent probe amplification (MLPA) are in routine use for predictive testing and have been evaluated in large patient series [73, 74]. Microsatellite analysis (MSA) and single-nucleotide polymorphism (SNP) arrays offer the additional opportunity to identify loss of heterogeneity (LOH) and detect isodisomy of chromosomes which might be missed by the other techniques. Using SNP data, an attempt at creating an evolutionary tree for uveal melanoma has been published [75].

9.5.1 Chromosome 3

The loss of one copy of chromosome 3 (monosomy 3) is the most frequent and prognostically most important chromosomal aberration in uveal melanoma and can be found in 50–61% of tumors [72, 76–81]. In 1996, a dramatic reduction in the 3-year survival probability from approximately 100% to less than 50% was reported for the first time in patients with monosomy 3 melanoma [72]. Since then, this finding has been confirmed by other authors several times [46, 55, 60, 82]. Only about 5–20% of patients with disomy 3 melanoma are expected to eventually develop metastatic disease [83]. Several possible explanations why metastasis occurs in a disomy 3 tumor have been discussed. First, intratumoral heterogeneity may lead to sampling errors if only a single small biopsy is used for analysis. The risk for misclassification with fine needle aspiration biopsy (FNAB) has been estimated to be less than 1% and however has to be considered [78, 84]. Second, e.g., FISH, CGH, and MLPA cannot detect loss of heterozygosity (LOH) caused by isodisomy and give the impression of metastasis occurring without monosomy 3. Partial deletions of chromosome 3 might also be missed by FISH. However, the prognostic significance of partial deletions is still unclear. Identified deletions affected regions of the short arm (3p11–3p14, 3p25–3p26, 3p25.1–3p25.2), as well as smaller regions on 3q (3q13–3q21 and 3q24–3q26) [85–88]. A critical region of deletion causing metastatic disease has not been identified, until Harbour et al. identified mutations of BAP1 gene located on chromosome 3p21.1 in the majority of metastasizing uveal melanomas by exome sequencing [45]. Loss of chromosome 3 seems to be an early event in development of uveal melanoma and can be found in combination with other chromosomal aberrations [89, 90]. Unmasking of the second allele with mutated BAP1 induces tumor progression and also determines an aggressive tumor phenotype [56]. It is therefore not surprising that the loss of chromosome 3 is associated with other predictors of a poor prognosis, such as increased tumor diameter, ciliary body involvement, epithelioid cell type, and extravascular matrix patterns [55, 72, 91, 92].

9.5.2 Chromosome 8

Aberrations of chromosome 8 may affect both the short and the long arm. Depending on the technique used, gains of 8q can be found in 35–75% and loss of 8p in 15–30%

[76, 93]. Gains of 8q and losses of 8p may occur together by formation of an isochromosome [90, 94, 95].

As for chromosome 3 changes of chromosome 8 are also associated with poor prognosis. Combining information on chromosome 3 and on chromosome 8 status improves the accuracy of prediction of metastatic disease compared to monosomy 3 or chromosome 8 status alone [46, 60]. Monosomy 3 and 8q gains occur together in about 45% of tumors and are associated with large tumor size, ciliary body involvement, and aggressive histology [80, 82]. In addition, chromosome 8 abnormalities are found in virtually all metastases from uveal melanoma, either to the liver or the brain [71, 94, 96]. Because of this and the variable copy number of 8q in one tumor, gains of chromosome 8 are thought to be a secondary event in uveal melanoma development. Improved analysis technique showed 8q amplification to be far more common than previously thought and high-resolution CGH identified frequent partial deletions on chromosome 8 [93]. Gains could be localized to 8q23–8q24 in many cases, and several oncogenes in that region have been evaluated for the potential significance. MYC, located on chromosome 8q24 and coding for a transcription factor, DDEF1 (development and differentiation factor 1), enhancing the motility of uveal melanoma cells, and NBS1 (Nijmegen breakage syndrome 1) were all analyzed for their possible role in promoting metastasis [97–100]. Though frequently overexpressed in uveal melanoma, an association to prognosis could not be established. ENPP2, also located on 8q24 and coding for autotaxin or ectonucleotide pyrophosphatase/phosphodiesterase family member 2 (ENPP2), has been identified by gene expression profiling as a predictor of survival [101]. Autotaxin is an enzyme producing lysophosphatidic acid (LPA), a small motility-enhancing and angiogenic lipid molecule [102]. Its role for tumor progression and prognosis in uveal melanoma still needs to be established.

Losses of chromosome 8p have also been analyzed in detail to identify possible tumor suppressor genes. Deletions could be localized on 8p12–8p22 by array CGH, and silencing of LZTS1 on the retained hemizygous allele was found [103]. This tumor suppressor genes code for the leucine zipper tumor suppressor-1 and inhibit motility and invasion of uveal melanoma in cell cultures. Interestingly, loss of 8p correlates with the prognostically unfavorable class 2B on gene expression profiling, further emphasizing the importance of 8p loss [89, 104].

9.5.3 Chromosome 6

Gains of chromosome 6p are observed in 28–54% of uveal melanomas and losses of 6q in 35–37% [76, 105]. Gains of 6p seem to be associated with a good prognosis. Possible explanations are that 6p gains are preferably found in spindle cell melanomas and rarely occur together with monosomy 3 [90, 106, 107]. Gains of 6p also correlate with gene expression profile subclass 1b, which is associated with a good prognosis. The protective effect of 6p gain might result from an alternative molecular pathway in tumor development dividing UM in two separate subgroups with good (6p gain) or worse (monosomy 3) prognosis. Loss of 6q, on the other hand, has been associated with a metastasizing phenotype. The effect of simultaneous 6p gain and 6q loss caused by chromosome rearrangements is unknown [77, 80].

Some authors have tried to identify tumor suppressor genes and oncogenes on chromosome 6; however, the genetic mechanism underlying chromosome 6 abnormalities remains unclear [108–110].

9.5.4 Chromosome 1

About a quarter of uveal melanomas show deletion on the short arm of chromosome 1, indicating a poorer prognosis. Because deletions of 1p are frequently found in larger tumors and in association with monosomy 3 and changes of chromosome 8, they are thought to develop later in tumor progression [78, 80, 84, 94, 111, 112]. However, 1p loss adds prognostic information independent of chromosome 3 status [112].

Several genetic mechanisms could be affected by 1p deletion, e.g., the NOTCH pathway and TP73 [113].

9.6 Molecular Genetics

9.6.1 MYC

The MYC gene is located on chromosome 8q24, a region frequently amplified in uveal melanoma, and therefore has been supposed to play a role in tumor progression [76, 90, 94, 95]. It codes for a transcription factor regulating the expression of numerous genes, controlling the cell cycle and apoptosis, and modifies the chromatin structure via histone acetyltransferases (HATs) [114, 115]. Many tumors show constitutive overexpression of MYC, which has also been identified in uveal melanoma and proposed as a potential prognostic marker [100, 116]. In cutaneous melanoma, overexpression of MYC seems to be associated with poor prognosis in cutaneous melanoma [117]. In uveal myeloma MYC is also frequently overexpressed but surprisingly associated with improved prognosis [97, 100]. Hence, the role of MYC overexpression in uveal melanoma remains unclear.

9.6.2 TGF- β

TGF- β 1 is located on chromosome 19, a region without frequent structural abnormalities in uveal melanoma. It has become of interest, because intraocular melanomas are growing in an immune privileged location, and the immunosuppressive properties inside the eye are mediated by cytokines, such as TGF- β [118]. TGF- β has antiproliferative and anti-apoptotic effects on various cell types (epithelial, endothelial, neuronal, leukocytes) [119]. TGF- β is secreted into the extracellular matrix and stored until its activation by numerous often unspecific stimuli, like as irradiation, reactive oxygen, and proteases such as plasmin and metalloproteinases [120, 121]. Metalloproteinases (MMP) have been of interest as a marker for tumor progression as they are involved in tumor invasion, angiogenesis, and metastasis, and TGF- β enhance and modify these effects [122, 123]. Whereas MMP-9 is

predominantly expressed in epithelioid melanomas and associated with poorer prognosis, immunohistochemical staining showed TGF- β positivity in uveal melanoma regardless of cell type, tumor size, or location [118]. TGF might also play a direct role in hematogenous metastasis to the liver, by increasing adhesion of uveal melanoma to the hepatic endothelium [124]. For metastases to grow to detectable size, induction of angiogenesis is a crucial step in tumor progression [125]. By its angiogenic effects, TGF- β could further promote growth of uveal melanoma metastases, and expression of TGF- β receptor endoglin has been found to correlate with metastatic death [126, 127]. A high number of tumor infiltrating leukocytes and macrophages as well as high HLA class I and II expression have also been linked with bad prognosis, as those are preferably found in monosomy 3 melanomas [128]. By downregulation of MHC class I antigen, on the other hand, TGF- β renders uveal melanoma cells more susceptible to cytolysis by natural killer cells [129, 130]. It has been suggested that uveal melanoma might prepare its own microenvironment for growth by secretion of local factors into the vitreous and aqueous humor [131].

9.6.3 Bcl-2

The Bcl family of proto-oncogenes comprises pro-apoptotic proteins, like Bax, Bad, and Bak, and anti-apoptotic proteins, like Bcl-2, Bcl-xL, and Bcl-w [132]. Bcl-2 seems to be of specific importance in regulation of tumor cell survival and apoptosis. As the pro-apoptotic members, Bcl-2 resides on the outer membrane of the mitochondria, inhibiting the initiation of the apoptotic cascade by the intrinsic pathway [133, 134]. Bcl-2 is strongly expressed in uveal melanoma, as shown by immunohistochemistry [97, 135–137]. Uveal melanoma is known to be resistant against radiation, and high radiation dose has to be used for the treatment of primary tumor. It is also resistant to standard chemotherapy. This is explained by the anti-apoptotic effects of Bcl-2. Therefore Bcl-2 has become a possible starting point in the search for a targeted therapy [138, 139]. Bcl-2 inhibitors could revert the inhibition of apoptosis and allow for the initiation of apoptosis by pro-apoptotic members of the Bcl family. Nemati et al. showed increased response of uveal melanoma to chemotherapy with fotemustine after the administration of a Bcl-2 inhibitor in animal xenografts [140]. Until recently no Bcl inhibitor with acceptable toxicity was available for use in humans [141, 142]. However, novel Bcl-2 inhibitors with low toxicity have been approved for use in chronic lymphocytic leukemia (CLL) with 17p deletion (affecting p53), and clinical testing in uveal melanoma is expected.

9.6.4 P53

P53 plays an important role as a tumor suppressor, inactivated in more than 50% of all tumors. The gene for p53 is located on chromosome 17p13.1. It is a transcription factor controlling the expression of other downstream genes to regulate cell proliferation and growth. Upregulation of p53 has been described in uveal melanoma

after irradiation [143, 144]. This appears plausible as p53 is a main factor in the cellular response to stress and DNA damage. Until DNA repair is completed, the cell is stopped in the G1 or G2 phase of the cell cycle [145]. If the damage is too severe to be repaired, the cell will undergo apoptosis. In contrast to other tumors, which frequently show altered expression or mutations of p53, the signaling pathway upstream of p53 seems to be intact in uveal melanoma [146]. However, there seems to be a functional inhibition of p53 in uveal melanoma. P53 interacts with several downstream molecules in a negative-feedback loop [147]. First, the cyclin-dependent kinase inhibitor CDKN2A stabilizes p53 by degrading Mdm2 through p14(ARF) [148, 149]. Second, it induces the expression of Hdm2/Mdm2 (human/mouse double minute 2). Overexpressed Hdm2/Mdm2 in reverse represses p53 transcriptional activity and also enhances its degradation [150–152]. This mechanism seems to be important in uveal melanoma, and overexpression of Mdm2 has been shown to be of prognostic value [143, 153]. Some authors evaluated the possible therapeutic effect of inhibition of Mdm2 by a small synthetic peptide in animal models; however, this has never been evaluated in clinical trials [141, 154, 155].

9.6.5 Rb

The Rb pathway has been shown to be frequently altered and functionally inhibited in uveal melanoma [143, 156–158]. To arrest cells in the G1 or G1/S phase, the Rb protein has to be kept in a hypophosphorylated state. This is accomplished by the cyclin-dependent kinase inhibitor CDKN2A coding for p16(INK)4a, keeping Rb active. In this hypophosphorylated state, Rb binds E2Fs, thereby repressing their transcriptional activity. Phosphorylation of Rb by cyclin-dependent kinases (CDK) can occur at multiple phosphorylation sites in Rb throughout the cell cycle, which gradually inhibits Rb function and releases E2F [159, 160]. Loss or inactivation of Rb will result in deregulated cell cycle progression and cell proliferation. In uveal melanoma Rb protein is frequently hyperphosphorylated and inactivated because of cyclin D1 overexpression and CDKN2A promoter methylation [143]. Still, most tumors, including retinoblastoma, need additional mutations of other tumor suppressor genes to develop [161].

9.6.6 Ras-Raf-MAPK Pathway (GNAQ, GNA11, CYSLTR2, PLCB4)

Mutations of proteins in the mitogen-activated protein kinase pathway (MAPK) have long been implicated in the development of conjunctival melanomas [162–164]. BRAF mutations are found in more than 65% of cutaneous melanoma, resulting in a constitutively active protein leading to MAPK activation [165]. Such BRAF mutations are rarely observed, on the other hand, in uveal melanoma [166–169]. Activation of the MAPK pathway has been demonstrated to be a frequent event in UM; however, this does not seem to occur through mutations of BRAF [170]. The mechanisms behind the activation remained obscure until the discovery

of mutations in GNAQ and GNA11 in uveal melanocytic lesions. A connection between mutations in GNAQ/GNA11 and proliferation of melanocytes was first established in 2004 during the analysis of determinants of skin color in mice [171]. Thereupon those mutations were found in almost 85% of uveal melanomas [20, 172, 173]. GNAQ and GNA11 encode the alpha subunit of heterotrimeric g-proteins (g-alpha-q and g-alpha-11). G-protein-coupled receptors (GPCR) are a large group of transmembrane receptors exerting multiple physiologic functions, such as in the sensory system, vision, olfaction, and taste [174]. Their function is transmitted by g-proteins from the extracellular to intracellular space. In their basal and inactive state, the alpha, beta, and gamma subunits are bound together with GDP. Upon activation through ligand binding to the g-protein-coupled receptor, the heterotrimeric g-protein dissociates and exchanges GDP to GTP [175]. The GTPase activity of the alpha subunit hydrolysis GTP to GDP. Mutations in the alpha subunit of GNAQ and GNA11 keep them in the activated state, leading to constitutive MAP-kinase pathway activation and cell proliferation without further extracellular stimuli. This raised the hope for targeted therapy with MEK inhibitors. In fact, a randomized open-label phase II clinical trial comparing selumetinib versus chemotherapy showed for the first time prolonged progression-free survival in patients with uveal melanoma metastasis [176]. However, this has not been confirmed in later studies [177].

In patients without detectable mutations in GNAQ/GNA11 of the g-protein-coupled receptor *CYSLTR2* and *PLCB4*, the gene encoding for the 1-phosphatidyl inositol-4,5-bisphosphate phosphodiesterase beta-4, a downstream target of GNAQ/GNA11, could be identified, further emphasizing the importance of MAP-kinase pathway activation through this mechanism [178, 179].

9.6.7 BAP1

Enormous efforts have been made to identify the critical mutation on chromosome 3 that promotes metastasis. Harbour et al. identified mutations of BAP1, located on chromosome 3p21.1, in 47% of metastasizing monosomy 3 uveal melanomas [45]. Interestingly, one of their patients had a germline mutation which was uncovered by loss of the second allele (monosomy 3). Soon after germline BAP1 mutations were found to cause a hereditary tumor predisposition syndrome, the spectrum of this is still evolving [40, 41, 180]. The BRCA1-associated protein-1 (BAP1) was first described by Jensen et al. as a potential tumor suppressor gene [181, 182]. BAP1 encodes a deubiquitinating enzyme and acts on BRCA1, histone H2A, host cell factor-1, and O-linked N-acetylglucosamine transferase (OGT) [181]. RNAi-mediated depletion of BAP1 in uveal melanoma cells resulted in loss of differentiation and gain of stemlike properties, similar to GEP class 2 tumors [56]. Though the molecular mechanisms of BAP1 are still not fully understood, a targeted therapy seems possible. Histone deacetylase (HDAC) inhibitors might restore histone H2A function which has been shown to be accompanied by increased melanocytic differentiation [183, 184].

9.6.8 SF3B1 and EIF1AX

Recently, additional somatic mutations have been identified in UM by exon sequencing, specifically occurring in patients with disomy 3 and partial monosomy 3 [185, 186]. SF3B1 is found in about 15–20% of uveal melanomas and associated with a favorable prognosis and prolonged metastasis-free interval compared to uveal melanomas with BAP1 mutations [186–189]. Mutations were mutually exclusive with BAP1 mutations and seem to support the bifurcated model of tumor progression in uveal melanoma [108, 190]. However, within the disomy 3 and partial monosomy 3 tumors, uveal melanomas with SF3B1 mutations had a worse prognosis than those without this mutation [189]. Interestingly, the mutational spectrum in SF3B1 of tumors with and without metastasis was different, and a further subgrouping of tumors according to mutation type might help to identify patients at risk of metastatic disease [186]. Tumors harboring EIF1AX mutations also are associated with a longer disease-free survival and rarely demonstrate metastasis [189]. Both mutations EIF1AX and SF3B1 are associated with good prognostic features, such as disomy 3; spindle cell type, positive BAP1 immunohistochemistry staining; and the absence of closed vascular loops [191].

9.6.9 Gene Expression Profiling (GEP)

While over the past decades methods for detection of cytogenetic changes and mutations have evolved and allowed for more detailed analyses, some other groups chose another approach offering a different view of the tumor and its microenvironment. Gene expression profiling (GEP), based on mRNA signature, has been described as a functional snapshot of the tumor microenvironment, which is perhaps less variable across the tumor and therefore less affected by intratumoral heterogeneity [192]. GEP was studied first in uveal melanoma cell lines, comparing those to normal melanocytes [193]. Then, Tschentscher et al. developed oligonucleotide microarrays to describe and compare the expression profile of monosomy and disomy 3 melanomas [194]. They also classified uveal melanomas according to their gene expression profile.

Soon afterwards, Onken et al. improved this technique and, leaving chromosomal status behind, classified uveal melanomas based on results from GEP, using unsupervised clustering methods to divide uveal melanomas in class 1 melanomas with low risk and class 2 tumors with a high risk of metastasis. Since then, numerous other investigators have compared the accuracy of GEP versus cytogenetic prognostic indicators. Unfortunately, as DNA-based techniques continuously evolved (MLPA, a-CGH, and SNP array), no direct comparison of GEP classification versus latest DNA-based techniques has been made. Onken et al. reduced the number of genes necessary for classification and developed a PCR-based microfluidics platform for routine clinical testing [192, 195].

Gene expression profiling closely correlates with cytogenetic findings in most cases [196, 197]. Class 1 tumors usually show disomy 3 and spindle cell type melanoma, whereas class 2 profile is associated with epithelioid cell type, monosomy 3. A further subclassification is possible into four groups (1A, 1B,

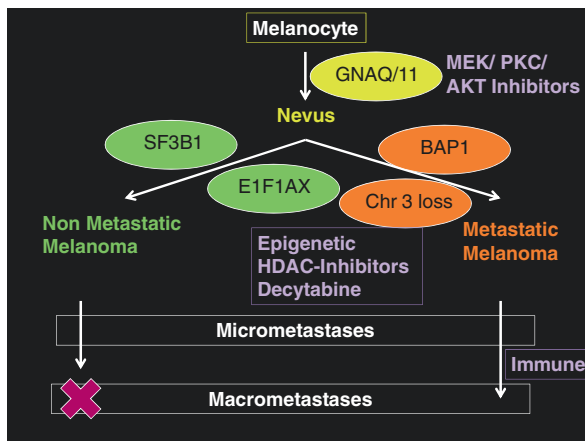


Fig. 9.1 Proposed bifurcated tumor progression model. GNAQ/GNA11 mutations induce growth of uveal nevi, which subsequently evolve into melanoma by acquiring (a) mutations in SF3B1 and/or EIF1AX, associated with a rather good prognosis, or (b) mutations in BAP1 and loss of the second allele by monosomy 3, which is associated with a high risk for metastasis. A more advanced model of an evolutionary tree for uveal melanoma was published by Nakul Singh et al. [75]

and 2A, 2B). Class 1A predicts the best prognosis, and class 1B is slightly worse, corresponding to disomy 3 melanomas with 6p gain. For class 2 melanomas, additional loss of chromosome 8p corresponds to the subclass 2B with the worst prognosis [89]. Based on this, a modified bifurcated tumor progression model has been proposed which is shown in Fig. 9.1. Some problems, however, remain. First, the significance and predictive value of GEP after radiation has not been evaluated so far and, as in contrast, has been done for DNA-based tests [198–201]. Also biopsy of non-melanoma tissue will reveal class 1 profile. For DNA-based tests, identification of GNAQ/GNA11 mutations can easily be done which will be positive in 85% of cases and ensure that melanocytic tissue has been biopsied.

9.7 Conclusion and Future Prospective

New genetic techniques have provided new and sometimes unsuspected insights into the pathobiology and the molecular carcinogenesis of uveal melanoma. Extended use of next-generation sequencing and proteomics will provide further insights, and targeted therapies will hopefully lead to an improved prognosis for patients with metastatic disease. Still, one should not forget to consider and reconsider again basic facts of melanogenesis in the light of latest findings as explained in the introduction of this chapter. It is still unknown whether choroidal nevi are congenital and slowly grow to clinically detectable size during life or whether they arise from normal melanocytes, which acquire their first transforming event (mutation of GNAQ/GNA11) later in life.

Findings from clinical syndromes like the oculodermal melanocytosis still pose many questions to be answered. Unifying concepts of pigment cell distribution and ocular melanogenesis may answer some questions but even more arise.

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