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Towards Liver: Selected Aspects of Uveal Melanoma Metastasis

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Abstract

Background: Uveal Melanoma (UM) although a rare disease, causes high mortality due to metastases. Long-term prognosis may be estimated based on the genetic profile of the UM tumor, however, lack of effective and specific treatment prevents cure.

Methods: This review summarizes current knowledge and outlines future directions relevant to UM metastatic disease.

Results: The overall consensus is that UM micrometastases remain dormant for a number of years, before actively proliferating and becoming detectable clinically. The main site of UM metastases is the liver, constituting a very specific niche, and numerous molecular factors are involved in UM liver homing, like CXCL12-CXCR4.

Conclusion: New avenues of research must include the mechanism of UM cells seeding from the primary tumor, homing to the liver, liver invasion, as well as UM cell dormancy.

Keywords: Uveal melanoma; Metastasis; Liver niche; Dormancy

Introduction

Melanoma is a relatively rare tumor originating from melanocytes and developing in various locations: The skin and mucosa (of the nose, nasopharynx, lungs, stomach, intestines, vagina, rectum and urinary tract), the conjunctiva of the eye, uvea, eyelids, and orbit. Uveal Melanoma (UM) is a neoplasm thought to have developed from neoplastic melanocytes of the uvea [1,2].

UM is the most common primary intraocular neoplasms in adults. In the USA, the mean age-adjusted incidence is 5.1 per million [3,4]. The UM incidence in Europe depends on the latitude, and is higher in Northern Europe (≥ 8 cases per million in Norway and Denmark), in comparison with Southern Europe (two cases per million in Spain and Italy) [5]. UM very rarely runs in families. Single families have been reported to show germline BAP1 mutation in chromosome 3, which predisposes them to develop UM and other neoplasms [6]. UM is usually diagnosed in people in their 6th decade of life, except for iris melanoma which is identified at an earlier age due to its location, most typically in the 5th decade of life, usually 10 to 20 years earlier than ciliary body melanoma or choroidal melanoma [7].

Risk factors for the development of uveal melanoma include Caucasian ethnicity, light eye color (green or blue), dysplastic nevus syndrome, ocular melanocytosis and presence of germline BRCA1-Associated Protein 1 (BAP1) mutations [8-11].

UM represents about 85% of all ocular melanomas and is biologically distinct from Cutaneous Melanoma (CM) [12,13]. CM has been gradually increasing since the 1970s, whereas the incidence of uveal melanoma has remained stable for many years [4,5]. The TNM (Tumor-Node-Metastasis) staging system, developed by the American Joint Committee on Cancer (AJCC) was based on collated approximately 9000 UM cases across Europe [14]. Shields et al. [8] studying another large group of UM patients determined that metastatic disease developed in only 5% patients with small tumors up to 1 mm thick, in 10% patients with tumors up to 2 mm thick and in 30% patients with tumors 6 mm thick. Up to 50% of patients develop metastatic disease (dissemination of UM), which are most frequently located in the liver.

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Long-term Prognosis in UM is Determined by Metastasis

It is well known that the life expectancy of UM patients is independent of the local tumor control and results from the development of metastases. The 10-year survival rates are 57% and 15-year survival rates are 50% [15]. These numbers have remained unchanged for many years, despite the improvements in the treatment of the primary disease [16]. This is because it is the progression of the disease in the liver that determines the clinical course of uveal melanoma.

The time-course of metastatic detection is varied and may even take a few years, at 5 years is estimated to be 32%, at 15 years 50% and 25 years 56 % [15]. Lower metastatic rates have been found in younger patients [17].

Metastases from uveal melanoma appear in 8% to 32% of the patients during the first 5 years and in 50% of the patients at 10 years after diagnosis of the primary tumor [15,17,18]. When liver metastases develop, the prognosis is poor and life expectancy reduces to 6 to 11 months and only 15% of patients are alive after more than 1 year [19,20].

The liver is the first site of systemic metastasis for 89% to 95% of patients and the exclusive site of systemic metastasis in more than 50% of patients. In approximately half of the cases also the lungs (24%), bone (16%), and skin (11%) may be involved. Very rarely metastases are found in lymph nodes (10%) and brain (5%) [21-24].

Recurring uveal melanoma patients' median survival time is less than 6 months from metastasis detection, regardless of treatment, and the one-year survival rate is estimated to be 10% to 15%. In contrast, a longer survival of about 19 to 28 months was found in patients with metastatic melanoma restricted to extra hepatic sites, and 76% of the patients survive over a year [25,26]. The latest deaths due to UM metastases occur between 10 and 18 years after diagnosis, but metastases after 40 years were also reported [15]. TOOT study provided evidence that local recurrence significantly increases the risk of metastasis despite the type of primary tumor treatment [27].

The mortality pattern of UM patients, regardless of treatment, presents a characteristic bimodal time-course with 1st peak at 2 to 4 years, and the second at 8 to 9 years after treatment [28]. A small percentage of patients have a delayed recurrence, at more than 10 years, and this correlates with longer survival [26]. It seems that these tumors grow more slowly. The mortality due to metastasis strongly correlates with the size of the tumor, as the percentage of deaths due to metastatic disease increased from 15% to 82% with the increase in basal diameter from less than 10 to greater than 18 mm [29].

The high mortality rate results from the lack of effective treatments for metastatic disease. Available options include resection of liver or chemotherapy. The general response rate for chemoembolization in uveal melanoma patients was 36%, compared with less than 1% in those treated with systemic chemotherapy [30]. Systemic chemotherapy is usually unsuccessful in metastatic uveal melanoma and results in an objective response rate that ranges from 5% to 15% [20].

The percentage of patients with metastases depends very strongly on the tumor thickness and its cytogenetics or genetic profile. Metastases were detected at 10 years after diagnosis in 12% of patients if the tumor was <3 mm, in 26% of patients of the tumor was 3 mm to 8 mm, and in 49% if the tumor was >8 mm [31].

An increased risk of metastasis and a poor prognosis in UM is connected with loss of one copy of chromosome 3 (monosomy 3, M3) [31]. Other chromosome abnormalities, amplification of chromosome 8q, loss of chromosome 1p, and gain of chromosome 6p, have been identified as prognostic parameters in UM [32]. Increased copy number of 8q precedes the loss of chromosome 3 [33]. Adding chromosome 3 and 8 status to AJJC grading of UM allows for more accurate prognostication [34,35]. Another approach is a 12-gene microarray-based gene expression panel to determine whether a patient is in a low- or a high-risk prognostic group [36].

Recently, a comprehensive genome, RNA, proteomics, and immune infiltrate analysis identified 4 distinct subsets of UM, i.e. (i) D3-UM tumors with EIF1AX mutation (low risk) or (ii) D3-UM with SF3B1 mutation (intermediate risk), correlated with distinct DNA methylation and SCNA profiles. D3-UM tumors also separated into two groups by transcription (mRNA, lncRNA, and miRNA) profile analysis. The other two groups (iii) and (iv) were M3/BAP1 (with biallelic BAP1 loss) with poor prognosis. M3/BAP1 aberrancy is associated with a global DNA methylation profile that is not observed in D3-UM. Despite all M3/BAP1-aberrant UM sharing this common DNA methylation pattern, these tumors divide into two groups by SCNA and transcription profiles, with distinct pathway features indicative of hypoxia, DDR, MYC/MAX signaling, and proliferation. The group M3/BAP1 loss group with the worse prognosis is characterized by the up-regulation of hypoxia, DNA damage repair, MYC, and down regulation of MAPK/PI3K, FOXA1/M1 and E2F1 pathways [37].

Most UM harbor one Gq pathway mutation (GNAQ, GNA11, CYSLTR2, or PLCB4), one BSE mutation (BAP1, SF3B1, or EIF1AX), and a few recurrent copy number aberrations, in 100% of tumor cells. These canonical changes usually occur relatively early in the tumor development, suggesting that metastatic abilities of the tumor may be determined early, maybe even before the detection of the primary mass. This would explain the lack of improvement in survival rates despite advances in diagnosis and treatment [38]. The genetic background of UM also determines the inflammatory environment, with gain of chromosome 8q leading to macrophage infiltration, while sequential loss of BAP1 expression drives T cell infiltration [39].

There is a very limited array of management options for patients with metastatic uveal melanoma. In a few cases, liver resection is possible. The available options have been reviewed in [21,40,41]. Local chemotherapy of different drug combinations has been applied with poor results to date [21,42-44]. The interim results of a clinical trial in immunotherapy, based on adoptive transfer of autologous tumor infiltrating lymphocytes in patients with metastatic uveal melanoma seem to be promising [45]. Other immunotherapy options are being explored [46,47]. High hopes for CTLA4 and PD-1 checkpoint immunotherapy so far have not been confirmed [48], however, recently a strong response with metastasis burden decrease was observed in a single UM patient with an MBD4 mutation [49]. This might open up a new avenue of research for effective immunotherapy in UM metastatic disease. There are several clinical trials enrolling subgroups of metastatic UM patients.

UM Metastases to the Liver

UM developing in the choroid spreads hematogenously mainly to the liver (Figures 1 and 2A-2D). Hematogenous metastatic

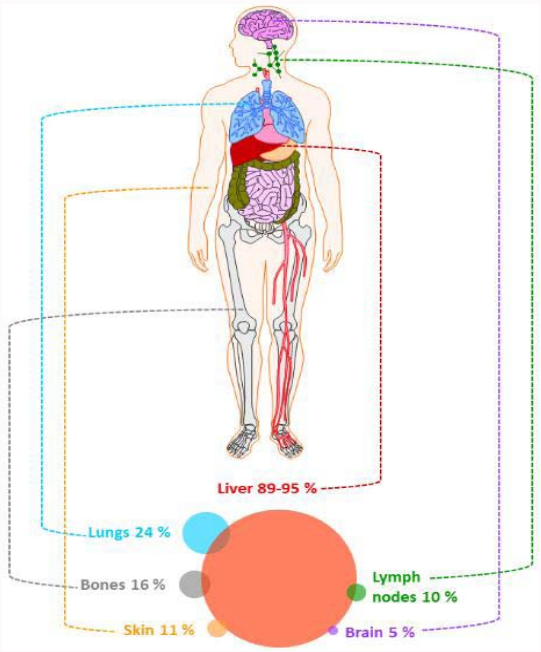

Figure 1: UM metastases appear in 50% of patients within 10 years of diagnosis of the primary tumor. The liver is the preferred metastatic site (89% to 95%). In approximately half of the cases also the lungs (24%), bone (16%), and skin (11%) may be involved. Very rarely metastases are found in lymph nodes (10%) and brain (5%).

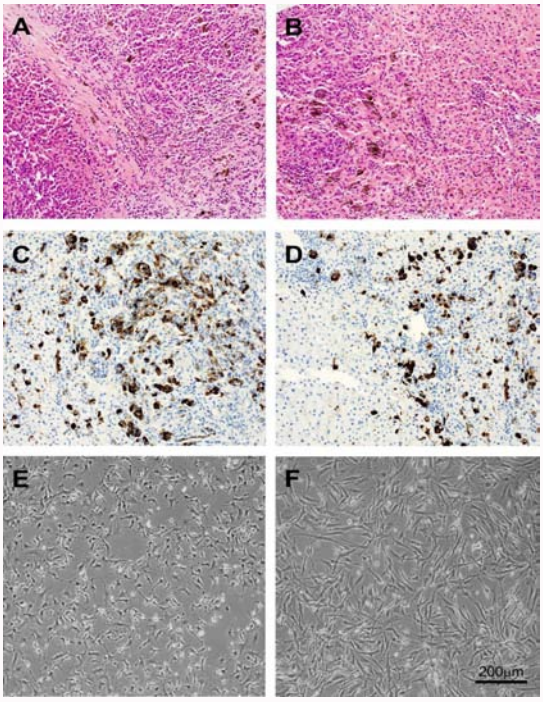

Figure 2: (A,B) Histology of a liver uveal melanoma metastasis. Uveal melanoma cells are seen invading normal liver. (C,D) Immunohistochemistry against HMB45 (brown) confirming presence of uveal melanoma cells in the liver lesion. (E,F) Cell lines derived from a single UM liver metastasis showing different cell morphology: Cells UM15.4.1 of epitheloid character (E) and UM15.6.3 of spindle shape (F).

spread is a multi-step process, including arrest by size restriction in micro vessels, rapid extravasation, and perivascular positioning, followed by single-cell dormancy or growth to micro metastatic and macro metastatic stage [50]. UM cell lines did not show a high pro-hemangiogenic potential, suggesting that other factors like the anatomy of ocular lymphatics seem to be responsible. The extraocular conjunctiva and limbus are well-endowed with lymphatic vessels, and the inner eye is physiologically devoid of these vessels. Therefore only when UM has grown outside the eye, may the cells find their way into the lymph system [51].

Romanowska-Dixon et al. [52] described the routes of extraocular extension *via* emissary channels of the sclera, like muffs surrounding vessels or other perforators (aqueous channels, ciliary arteries, ciliary nerves, optic nerve, vortex veins). All 'locus minoris resistances" make extraocular invasion of UM cells easier, regardless of the cell type [52]. They have shown the routes of extension without sclera invasion or with lamellar sclera infiltration in the group of 170 patients with intraocular tumor size between 1.5 mm to 15 mm. The extrascleral extension was detectable with ultrasound/ultrabiomicroscopy before enucleation in 18% of patients with extraocular tumor invasion [53].

Damato pointed out the many possible hypotheses formed on how metastases develop in UM and whether or not the treatment influences survival. He proposed that UM are consisting of at least three groups: (1) metastasizing melanomas, which have already metastasized by the time of ocular treatment even though the metastases may not be detectable; (2) pre-metastasizing melanomas, which develop metastatic capability and disseminate if treatment is delayed and (3) non metastasizing melanomas, which do not metastasize, even if never treated [54]. In the light of the dormancy concept however, one may speculate that all UM are in the first group, but that in some cases the metastases remain dormant for many years.

It is believed that micrometastatic disease precedes local therapy, remaining dormant for a long time. Moreover, there is a correlation between metastatic risk and the size of the tumor [29], suggesting that metastatic seeding might occur over the whole time of the tumor growth. Therefore the results of Damato et al. [29] from analyzing a large group of patients pointing out that early treatment of UM should prevent metastatic spread in some patients are in accordance with this hypothesis.

Eskelin et al. [55] estimated that micro metastases from uveal melanoma could develop as early as 5 years before the treatment of the primary tumor. At this estimated time of micro metastases, the theoretically estimated size of the primary tumor would be app. 3 mm in diameter and 1.5 mm in height or only 7 mm 3 in volume [55].

The current concept in cancer cell dissemination is that there are several cancer cell migration modes, i.e. single cell, multi cellular streaming and cell clusters, containing both tumor and stromal cells [56]. Analysis of the structure of liver from UM patients revealed the presence of single cells, as well as micrometastases and macro-metastases [57-60]. Both single cells and cell clusters were detected in the blood of UM patients [61].

Grossniklaus et al. [57] have been studying the structure of liver metastases both in the animal model [62], and in the clinical samples [57,58]. They describe the hypothetical metastasis progression from single cells, having cancer stem cell-like characteristics. The presence of CD45/CD133 individual UM cells were shown in the sinusoidal space, portal venule and periportal area, suggesting they are the precursors to metastases [58].

The liver UM metastases result in two distinct growth patterns:

The infiltrative and nodular growth patterns. In the infiltrative pattern, melanoma invades the sinusoidal space, replaces the hepatic lobule, is essentially avascular, and does not express VEGF. These lesions generate MMP, leading to the creation of pseudosinusoidal spaces providing tumor oxygenation. In the nodular growth pattern, melanoma arises in the periportal area, coopts the portal venule, and eventually grows, becomes hypoxic, expresses MMP9 and VEGF, undergoes angiogenesis, and effaces the adjacent hepatocytes. The authors proposed that infiltrative growth is controlled in part by changes in the immune microenvironment in the sinusoidal space and nodular growth is controlled in part by the microenvironment VEGF: PEDF ratio in the periportal area [58]. UM cell aggregates have enhanced adhesion due to proinflammatory factors release, and later on activate hepatic stellate cells to myofibroblasts that form a scaffold for the metastasis to grow [57]. The same authors also attempted to correlate the histological results from biopsies with non-invasive MRI of liver lesions [63].

Some authors suggest that both perivascular and intravascular migration can contribute to the spreading of metastatic cells in the target organ [50,60]. During liver colonization individual tumor cells initially migrate inside or along sinusoids until a critical local cell density is reached and growth initiates [50]. In another study, using international consensus guidelines the histopathological growth patterns of liver metastases were studied in resected livers from UM patients [60]. For 41 liver metastases, 30 (73%) were classified as a predominant replacement pattern (where tumor cells were replacing liver cells), while 11 (27%) as a predominant desmoplastic pattern (tumor cells form a nodule, separated by a fibrotic tissue from surroundings). In a similar fashion to colorectal and breast carcinoma, the replacement pattern significantly predicted diminished survival while the desmoplastic pattern correlated with increased survival. The genomic high-risk variable had no prognostic value at this stage of liver metastasis. In the replacement pattern tumor cells occupy 'vascular niches', such as the space of Disse. What is interesting, the melanoma cells located in the vicinity of sinusoidal vessels were localized to the abluminal vascular surfaces of sinusoidal vessels and in the space of Disse, rather than being intraluminal [59,60]. These results might provide a prognostic marker for the metastatic patients if they could be related to radiologic parameters.

The role of hepatic stellate cells in creating the metastatic niche has been suggested [64]. The hepatic stellate cells may transdifferentiate into myofibroblasts and secrete proinflammatory factors and collagen. In the mouse xenograft model, the number of hepatic metastases was increased when human HSteCs were co-inoculated, leading to an increase in fibrillar collagen production. The presence of activated hepatic cells and their pathological matrix were also localized surrounding the UM lesions in patient hepatectomy samples [64].

The research on UM metastasis mechanisms and UM treatments efficacies are hampered by lack of appropriate UM models. Only a few stabilized cell lines and PDX are available [65-67]. An example of stabilized cell lines, derived from a patient metastatic biopsy is shown in Figure 2E and 2F.

Dormancy of the Disseminated Tumor Cells

Dormancy can be broadly defined as the process though which cells exit the cell cycle and survive in a quiescent state. It is considered an evolutionarily conserved mechanism of adaptation to stress which allows cells to survive in a hostile microenvironment [68]. Disseminated cancer cells are able to persist for years because they interpret homeostatic signals from the host microenvironment and respond by entering a long-lasting dormant state, with occasional cell divisions (cellular dormancy) [69]. Similarly, tumor cells may give rise to micrometastatic lesions that are unable to outgrow until they avert immunosurveillance and elicit a supportive angiogenic response (micrometastatic dormancy) [70,71]. Dormancy may also result from an equilibrium between proliferation and apoptosis that results in the equilibrium of a subclinical tumor mass (tumor mass dormancy) [68].

Regulation of cellular proliferation, autophagy, and modulation by metastasis suppressor genes as well as micro environmental cues, such as interaction with extracellular matrix, hypoxia, impaired angiogenesis, inflammation and immunity are implicated in dormancy control [68,69,72].

In cancers other than UM, NR2F1, TGFbeta2 or HES1 seem to be involved in inducing or prolonging dormancy [73]. The UPR was found to promote the survival of dormant cancer cells and it has been linked to how cancer cells respond both to internal (metabolic) stress and external stress (adaptation to foreign extracellular matrix composition [74]. Another important discovery was that the endothelium induced quiescence in breast cancer cells through the production of Thrombospondin-1 (TSP-1) but sprouting neovasculature induced disseminated cell proliferation mediated by the secretion of TGF-β1 and periostin (POSTN) by endothelial tip cells [68]. In metastatic spreading of lung and breast cancer cells in the perivascular niche it was found that L1CAM and YAP signaling enables the outgrowth of metastasis-initiating cells both immediately following their infiltration of target organs and after they exit from a period of latency [75].

Liver Metastatic Niche

Dormant disseminated tumor cells may reside in specialized niches that support their survival, restrain their proliferation and finally lead to their reawakening [70,76]. In this they are similar to cancer stem cells as they enter into dormancy and eventually undergo reactivation in response to niche signals similar to those that regulate normal adult stem cells [70,74]. For example, prostate cancer cells metastasizing to the bone have been found to compete with hematopoietic stem cells for occupancy of sites in the endosteal niche; this occurs via the CXCL12-CXCR4 signaling axis that is normally reserved for the physiologic regulation of hematopoietic stem cells [76]. Primary UM expressing high levels of c-Met and/or CXCR4 aggregate in the liver, which contains the c-Met ligand, HGF/SF and the CXCR4 ligand, SDF [58].

In fact, premetastatic niche might be "a sleepy niche", tightly regulating the dormancy of disseminated cancer cells [77]. Some cellular and molecular factors regulating the dormancy have been mentioned above. However, it is becoming clear that niche-based cues function only in the context of specific tissues. Another conclusion is that maintenance of tissue homeostasis is crucial. Most dormant breast cancer cells associate with the abluminal surface of the microvasculature of distant organ sites. As long as microvasculature is stable, it produces dormancy-inducing factors. Endothelial quiescence can be disrupted by e.g. inflammation, wounding and ageing, and then signals change to induce tumor cell proliferation [77].

Liver is the target organ for metastasis for many cancers - GI tract, breast, pancreas, lung, cutaneous melanoma and sarcomas and some progress in understanding the formation of the liver metastatic niche has been described. Many environmental factors have been shown to play a role, such as active bi-directional communication by mRNA in exosomes [78,79], fibroblasts [80], or hepatocyte progenitor cells [81].

Inflammation and immune cells are also factors influencing dormancy. Breast and lung carcinoma cells selected for their ability to persist in a latent state after seeding of distant organ sites succeed in evading clearance by NK cells through the repression of various NK cell-activating ligands, a program that appears to be tightly coupled with entrance into a quiescent state [76]. Recently Albrengues et al. [74] showed that bacterial-derived Lipopolysaccharide (LPS), a trigger of inflammation, or cigarette smoke (which may carry LPS as a contaminant) can activate neutrophils to release their DNA content into the lung parenchyma to form Neutrophil Extracellular Traps (NETs) that usually capture microorganisms. This activates focal adhesion kinase and induces proliferation in dormant DCC [74].

One characteristics of the liver microenvironment that may play role in the dormancy control is its immune microenvironment. Liver is known to facilitate immune escape [82,83]. Moreover, in the aged mice a slower growth of the ocular tumor was seen, but more liver metastases due to the lower cytolytic activity of NK cells in the liver, and bone marrow derived cells played a role in the heightened metastases [84].

The presence of residual UM cells in the bone marrow was detected in 39% patients at diagnosis. They were mostly vital melanoma cells, documenting that dissemination are an early event in uveal melanomas, supporting the dormancy hypothesis [85]. The quiescent state of dormant cells contributes to the observed resistance to conventional therapies aimed at targeting rapidly diving cells. The role of CTC as a negative prognostic marker was demonstrated in uveal melanoma patients after a long follow-up period. The number of CTC (lower or higher than 10 CTC per 10 mL blood) and the presence of CTC clusters correlated significantly with largest basal diameter, tumor height, and disease-free and overall survival [61]. Both CTC and ctDNA were found to be prognostic in another study. CTC count and ctDNA levels were associated with the presence of miliary hepatic metastasis, with metastasis volume, and with each other. CTC count and ctDNA levels were both strongly associated with progression-free survival and overall survival [86].

The exact mechanisms and all the molecular, cellular or microenvironmental factors responsible for maintaining dormancy or influencing reawakening of cells from dormancy in UM are unknown and require intense studies.

Cellular and Molecular Mechanisms Associated with UM Metastasis

There is a plethora of molecular factors and pathways shown to be connected to metastasis in UM. They are studied either as markers of a distant disease, and therefore potentially of prognostic value, or as a part of the mechanism responsible for metastatic spread. An excellent, detailed analysis of them is presented in other papers [87,88]. Several research directions mentioned below seem to be worth further studies, especially in the context of animal models.

For example, GNAQ stimulates the transcriptional co-activator YAP in human uveal melanoma cells. YAP/TAZ has been shown to act as stiffness sensors, regulating mechano-transduction, which is an important part of cellular motility. Disruption of the actin cytoskeleton diminished both the basal activity of YAP and YAP hyper activation [89]. GNAQ mutation also induces viability and migration of uveal melanoma cells *via* Notch signaling activation, which is mediated by YAP dephosphorylation and nuclear translocation [90].

Loss of BAP1 expression in UM tumors associated well with all of the methods currently used for prognostication and was itself predictive of death due to metastasis in uveal melanoma after enucleation [91]. Silencing BAP1 in 92.1 cells led to dedifferentiated phenotype, characteristic of more invasive class II tumors. BAP1 depletion also caused a reduction in mRNA levels of neural crest migration genes (ROBO1), melanocyte differentiation genes (CTNNB1, EDNRB and SOX10) and other genes that are down-regulated in class 2 tumors [11].

Cellular plasticity and stemness seem to play a role in UM, and therefore may be expected to be significant in UM metastasis as well, as biomarkers such as beta-catenin, E-cadherin, and hypoxia-inducible factor 1alpha most strongly associated with the more aggressive tumors [92]. BAP1 is necessary for maintenance of melanocyte identity in uveal melanoma cells, and that loss of BAP1 leads to a loss of cell identity and acquisition of a primitive, stem-like phenotype [93]. The studies using established UM cell lines (Mel270 and OMM2.5) revealed their heterogeneity and the presence of a CSC-like subpopulation with enhanced self-renewal and proliferative capabilities [94]. High cellular plasticity was also found in UM cells from short-term primary cultures. The authors concluded that inherent changeable phenotype of UM may be responsible for the fact that hierarchical CSCs have not been conclusively identified in UM [95]. CD133-positive cells, i.e. putative cancer stem cells were detected in uveal melanoma. What is interesting they were predominantly localized at the invading tumor front, which may be a further indication for the tumorigenic and metastatic potential of these cells. Other putative stem cell markers (Sox2, Pax6, Musashi, ABCB5) also predominantly localized to these areas [96].

Epithelial-to-Mesenchymal Transition (EMT) is a critical cellular event for metastasis of malignant tumors of epithelium origin and promotes mesenchymal phenotype, leading to intravasation of tumor cells into the blood stream or lymphatic vessels with the subsequent formation of distant metastases. UM is of neural crest origin, and clinically, spindle mesenchymal phenotype (spindle cell) of UM is indicative of less aggressive malignancy as compared to the aggressive epithelial phenotype. Expression of several EMT factors were studied in UM cell lines and tumor samples and it was shown that ZEB1 is highly expressed in uveal melanoma cell lines, while two other EMT factors, Twist1 and Snail1, were also expressed, but to a lesser degree. The genetic down regulation of these factors reduced the invasiveness of uveal melanoma cells *in vitro*, and ZEB1 and Twist1 mRNA levels significantly increased in primary tumors with high metastatic risk [97]. Chen et al. showed that spindle UM cells can convert to epithelioid UM cells both *in vivo* and *in vitro*. They pointed out that higher levels of ZEB1 propel UM progression by promoting cell dedifferentiation, proliferation, local migration and invasion, though had little effect on EMT morphology and concluded that ZEB1 is an oncogenic factor required for UM growth and metastasis.

Chemokine receptors and their respective ligands, e.g. CXCR4/CXCL12 is implicated in uveal melanoma metastases. High expression

of CXCR4 on UM cells facilitates the accumulation of uveal melanoma cells in the liver [98]. It was also shown that ocular microenvironment factors induce methylation and down regulation of tumor CXCR4 expression [99], so perhaps this chemokine role is not dominant in UM. Another molecule, c-Met, a receptor for Hepatocyte Growth Factor (HGF), promotes invasion and stimulates tumor growth through a paracrine effect produced by hepatocytes. High levels of soluble c-met were found in blood of patients with metastatic disease and suggested to be a possible marker [100]. Crizotinib, an inhibitor of c-met, significantly reduced development of metastases in a mouse model, suggesting that the inhibition of c-Met activity alone may be sufficient to strongly inhibit formation of UM metastasis [101].

Exosomes secreted by tumor cells are one of the paracrine signaling ways and research on their role in UM is just beginning. Exosomes isolated from liver perfusate of UM metastatic patients contained miRNA pattern characteristic of UM cells [102]. UM-derived exosomes expressing integrin α V/integrin β5 are taken up by liver-specific cells to prepare the premetastatic niches and steer the liver tropism of UM cells [103].

Hypoxia is one of the factors increasing tumor aggressiveness. An increase in Hypoxia-Inducible Factor 1a (HIF-1a) expression was seen in more than 60% of UM patients and was significantly associated with proliferative and vascular markers, as well as necrosis [104]. HIF-1a protein expression was increased in well-vascularized tumor regions as well as in four cell lines grown in normoxia. Growth in hypoxia significantly increased cellular invasion UM cell lines tested. Genetical or pharmacological blockade has negative effects on tumor growth and invasion, activation of Notch and MAPK was required for full induction of cellular invasion under hypoxic conditions [105]. In clinical UM samples an increased expression of HIF1a, and a decreased expression of VHL were associated with monosomy 3/ loss of BAP1 expression. The possible mechanism might involve an up-regulation of HIF1a due to increase in NF-kB expression with BAP1 loss. What is more, HIF1a was associated with the presence of macrophages and lymphocytes, also correlating with increased NF-kB [106].

Conclusion

Metastases remain the main challenge in uveal melanoma management due to lack of specific treatment. Despite a marked progress in uveal melanoma development has been made, the metastatic spread needs further intense research. For example, it is well established that the genetic profile of the UM tumors characterized by monosomy 3 and bilallelic BAP1 loss is associated with the worst prognosis. The growth stages of UM lesions in the liver were described, and many potential molecular factors influencing the process of metastatic spread and colonizing the liver niche are known, however our understanding of this process is far from clear. Critical questions on the mechanism of UM cells seeding from the primary tumor, homing to the liver, liver invasion, UM cell dormancy and dormancy ending need to be answered urgently. Only understanding many aspects of UM metastases, including its timeline, organ specificity, molecular biology will enable the new therapeutic opportunities.

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Compliance with Ethical Standards

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the Helsinki Declaration of 1975, as revised in 1983 and approved by Jagiellonian University Bioethics Committee (no: 122.6120.58.2016). Experiments on animals were approved by the I Local Ethics Committee for Animal Experiments (no 99/2015).

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