Introduction
to
Insights of Tumorigenesis and Metastasis of Uveal Melanoma

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Although considerable progress has been made in elucidating the biological mechanisms underlying uveal melanoma growth and dissemination, further investigation is required as there is still no effective treatment available for metastasis in uveal melanoma patients. Identification of genes, proteins and pathways involved in the metastatic disease has started to provide some insight in the dissemination of uveal melanoma. In order to be able to treat or prevent metastatic disease, development of novel anti-cancer strategies is urgent and requires more sensitive and less invasive methods, to detect and monitor in vivo tumor growth and metastatic disease in cancer models.

The main objective of this thesis is to develop a new model in which local tumor growth and metastatic disease can be studied and to explore tumor growth mechanisms of uveal melanoma.

### Uveal melanoma - Clinical aspects

Uveal melanoma is the most common primary malignant intraocular tumor in adults, with an annual incidence of 6–8 per million in Caucasians\(^1\). The incidence increases from the age of 30 up to the age of 70, with a peak incidence in the sixth decade\(^2,3\). Uveal melanoma develops in one of the most capillary-rich tissues of the body and has a hematogenous dissemination. The therapy of uveal melanoma remains problematic due to a high rate of metastatic dissemination irrespective of the success of treatment of the primary tumor. This suggests that dissemination and metastasis are early events during uveal melanoma development. There is no evidence that early detection of micrometastasis has led to improved survival. It is, however, important to note that formation of micrometastasis is the pathological basis for the occurrence of clinically overt metastasis. Therefore, development of therapeutic strategies aimed towards interfering with initial events in organ colonization and dissemination of metastasis is required.

Metastases are predominantly localized in the liver (87%), lungs (46%) and bone (29%)\(^4\). Upon diagnosis of uveal melanoma the patient’s median survival time is 6.5 years\(^5\). Once hepatic metastases have been diagnosed, the patient’s life expectancy is less than 7 months\(^4,6-8\).

### Diagnosis and treatment

Melanocytes are embryologically derived from the neural crest. In the mature eye melanocytes occur in choroidal stroma and provide its brown color (Figure 1). Melanocytes contain fine pigment granules (melanosomes), are 0.3-0.4µm wide, oval in shape, yellowish to darkbrown in color and smaller than those of the retinal pigment epithelium\(^9\). The diagnosis of uveal
melanoma can usually be made by recognition of the typical ophthalmoscopic features of the neoplasm (Figure 2A and B) 10.

**Illustration of the Eye**

Ancillary diagnostic studies as ultrasonography and fluorescein angiography are important aids in the diagnosis. In atypical cases, the use of sophisticated techniques such as magnetic resonance imaging and transocular fine-needle aspiration biopsy, may provide further diagnostic assistance 11-13.

The management of malignant melanoma of the posterior uvea has been a topic of great controversy 14-16. A number of authorities have challenged the traditional treatment by enucleation of the tumor-containing eye, and clinicians more frequently are using alternative methods of management when possible. Current management ranges from periodic observation and fundus photography of selected small lesions that appear dormant, to thermotherapy, plaque radiotherapy, beam teleradiotherapy, local resection or a combination of these treatment modalities 17,18. In case the tumor is far advanced (largest tumor diameter >16 mm and/or thickness >8mm) and there is no hope for eye saving treatment or useful vision, enucleation is often inevitable 19. Enucleation is performed in less than 20% of cases today. In cases with massive orbital extension, orbital exenteration might be necessary 20-23.

However, choosing the optimal therapy is a complex issue and treatment must be individualized in each case. In selecting a therapeutic approach, certain factors should be carefully weighed. These include the visual acuity and intraocular pressure in the affected eye, the size of the melanoma, its extent and location, scleral outgrowth and cytogenetic features, apparent melanoma activity, the condition of the opposite eye, age and general health of the patient,
the exclusion of distant metastasis (figure 3) and the psychological status of the patient.

Figure 2A: Picture of a uveal melanoma of the choroid(*) detected with funduscopy
Figure 2B: Picture of an enucleated eye with an uveal melanoma of the choroid (*)

Figure 3: Picture of multiple pigmented liver metastasis of a uveal melanoma. Reproduced with permission of Prof. Tollenaar, Department of Surgery, Leiden University Medical Center, the Netherlands.

Pathogenesis of uveal melanoma

Histologically, uveal melanoma cells have been classified as 1) spindle-shaped, 2) epithelioid or 3) mixed types. The epithelioid phenotype has been associated with poor prognosis and metastatic behavior. Well-known established prognostic factors are tumor thickness, diameter and localization, cell type, monosomy of chromosome 3 especially in combination with concurrent loss of chromosome 1, number of mitosis and vascular loops. Monosomy of chromosome 3 and a large tumor diameter are the most significant factors.
determining survival of patients. Monosomy 3 occurred in around 50% of cases. It has been suggested that this aberration plays an important role as initiator or as an early step in melanoma development.

**Oncogenes**

The c-myc oncogene locus has been found in 65% of uveal melanoma and an association was established with c-myc amplification and increased tumor size. Expression of c-met (proto-oncogene) was found in uveal melanoma cell lines, but the c-met ligand, hepatocyte growth factor/scatter factor (HGF/SF) was not expressed. Interestingly, uveal melanoma tissue with liver metastasis expressed HGF/SF most intense at the level of the choriocapillaris which may be a site of dissemination, suggesting that it may play a role in the metastatic process. The importance of oncogenic mutations in the RAS/RAF/MEK/ERK pathway has been well documented in human cancers. More than 15% of all human cancers harbor point mutations of RAS. Although several studies showed no mutations for this specific pathway, a recent report proved that BRAF (v-raf murine sarcoma viral oncogene homolog B1) is mutated in 50% of uveal melanoma.

**Tumor suppressor genes**

The Rb (retinoblastoma) gene and p53 transcription factor play an important role in several tumors like retinoblastoma and osteosarcoma, but no Rb or p53 mutations or selected genes in these pathways (p14, p15, p16, and TGF-β R2), have been found in uveal melanoma. Van der Velden et al., demonstrated that hypermethylation of p16 is a cause of inactivation of this gene, which interestingly was more frequently in tumors from patients with metastatic disease. However, uveal melanoma may contain functional abnormalities in the Rb and p53 pathways, rather than mutations in these factors themselves. For instance, expression of 15% or more of cyclin D1, an activator of Rb kinase and HDM2 (human homologue of murine double minute) protein, an inhibitor of p53 in uveal melanoma is associated with an unfavorable outcome.

**Dissemination and metastasis**

Blood vessels are assembled during embryonic development by vasculogenesis, which is a primitive vascular network is established from endothelial precursors. The process of blood vessel formation from pre-existing ones is known as angiogenesis or neovascularization. Angiogenesis is critical to chronic inflammation and fibrosis, to tumor growth, and to the vascularization of ischemic tissues. Tumors stimulate the growth of host blood vessels, which is essential for supplying nutrients to the tumor. Tumors cannot grow beyond 1 to 2 mm in diameter or thickness unless they are vascularized (see section 1.4 Angiogenesis and vasculogenic mimicry). Angiogenesis is a requisite not only for continued tumor growth, but also for metastasis. Without access to the vasculature, the tumor cells cannot spread to distant sites. Tumor blood vessels differ from the normal vasculature by being tortuous and irregularly shaped and being leaky. The leakiness is attributed largely to the increased
production of VEGF (vascular endothelial growth factor). Tumor cells may line structures that resemble capillaries, a phenomenon called vascular mimicry. In order to acquire motility and invasiveness, carcinoma cells must shed many of their epithelial phenotypes, detach from epithelial sheets, and undergo a drastic alteration: the epithelial–mesenchymal transition (EMT). Recall that the EMT involves a shedding by epithelial cells of their characteristic morphology and gene expression pattern and the assumption of a shape and transcriptional program characteristic of mesenchymal cells. This major shift in epithelial cell phenotype is necessary for the reconstruction of epithelial cell layers after wounding. The EMT is used even more widely in certain morphogenetic steps occurring during embryogenesis, when tissue remodeling depends on EMT executed by various types of epithelial cells. It is plausible that all types of carcinoma cells must undergo an EMT in order to become motile and invasive. The cellular changes associated with EMT encompass the loss of cytokeratin, E-cadherin expression and cell polarity. EMT also coincides with the acquisition of motility, invasiveness, vimentin, altered adhesion suppression and proteinase secretion. Invasion and metastasis are biological hallmarks of malignant tumors. The acquisition of an invasive phenotype through the expression of proteolytic enzymes provides mechanisms for uveal melanoma cell to overcome the physical constraints imposed on them by intercellular junctions and the extra-cellular matrix. The matrix metalloproteinase (MMP) family of enzymes is involved in the degradation of extra-cellular matrix components at the tumor-host tissue interface. This is a key event in the invasive process. MMP2 and MMP9 expression has been associated with metastasizing primary uveal melanoma and reduced survival.

The migration of neuroepithelial cells, like melanocytes, from the neural crest into the mesenchyme of early vertebrate embryos also depends on a transformation of cell phenotype that can best be described as an EMT. Expression of E-cadherin and cytokeratins - hallmarks of epithelial cell protein expression - is repressed, while the expression of vimentin, an intermediate filament component of the mesenchymal cell cytoskeleton, is induced. Epithelial cells that have undergone an EMT often begin to make fibronectin, an extracellular matrix protein that is normally secreted only by mesenchymal cells such as fibroblasts. At the same time expression of a typical fibroblastic marker - N-cadherin - is often acquired in stead of E-cadherin. These and alike observations indicate the involvement of certain heterotypic signals that originate in the reactive stroma of primary carcinomas, which impinge on neoplastic cells located at the outer edges of the epithelial cell mass, and induce these cells to undergo an EMT. N-cadherin is expressed in fibroblast and invasive carcinomas like uveal melanoma. A micro-array study showed that primary uveal melanoma can be divided into 2 classes: a group with low risk and an aggressive group with high risk for metastatic death. One of the genes discovered is Id2 (helix-loop-helix inhibitor), which was down-regulated in the aggressive group. Id2 seemed to suppress the epithelial-like phenotype by inhibiting the E-cadherin promoter. Abundant evidence indicates that transforming growth factor
(TGF) β is an important agent for conveying these stromal signals. TGFβ is known to be capable of suppressing the growth of normal human melanocytes but this response is lost in melanoma cells. TGF-β has a potential dual role in controlling uveal melanoma invasion: down-regulation of adhesion and up-regulation of protease secretion. Uveal melanomas could be regulated by both autocrine and paracrine stimulation of TGF-β. TGF-β can modulate a wide variety of immune reactions and it has been documented that TGF-β, at concentrations found in the aqueous humor of the eye, can significantly alter MHC (major histocompatibility complex) class I antigen expression and the susceptibility of ocular melanoma cells to natural killer cell mediated cytolysis. TGF-β may specifically regulate interaction of uveal melanoma cells with hepatic endothelium by promoting adhesion.

Another member of the TGFβ superfamily, Bone Morphogenetic Protein -7 (BMP7), has been shown to induce Mesenchymal-to-Epithelial Transition (MET) (and/or inhibit EMT) (see section 1.3 Bone Morphogenetic Protein family members in the TGFβ pathway).

Bone morphogenetic protein family members of TGFβ superfamily

the transforming growth factor β superfamily. Fifteen BMPs have been identified, which have the unique function of inducing bone formation. Although BMPs are synthesized by skeletal cells, their synthesis is not limited to bone because they are expressed by a variety of extraskeletal tissues in which they play a critical role in development and cell function. A fundamental function of BMPs is to induce differentiation of mesenchymal cells toward cells of the osteoblast lineage thus promoting osteoblastic maturation and function. As osteoblasts undergo terminal differentiation and the cellular matrix mineralizes, they undergo apoptosis.

Besides in skeletal development BMPs (and their antagonists) play a crucial role in organogenesis and postnatal development. Gene inactivation of various BMPs often result in significant phenotypic changes in various organs. For example: BMP7 null mice display lack of eye and glomerular development, leading to renal failure and neonatal death. These mice also have modest forms of skeletal abnormalities including fused ribs and vertebral or skull defects.

Furthermore, BMPs play an important role in the pathophysiology of several diseases including osteoporosis, arthritis, kidney diseases, pulmonary hypertension, cerebrovascular diseases and cancer. BMP signaling is precisely regulated by BMP antagonists. The interplay between BMPs and their antagonists governs developmental and cellular processes like establishment of induction of neural tissue, formation of the eye and formation of joints in the skeletal system. Mesenchymal cell lines, bone marrow cells, osteoblast precursors, myoblasts, fibroblasts and neural cells all respond to BMPs. Recent research shows a disparate role of BMP in the stem cell biology. Stem cells originating from neural crest tissue give rise to diverse cell types including uveal melanocytes.
These neural crest stem cells treated with TGFβ differentiate into specific cell types. BMPs have been shown to differentiate mouse embryonic stem cells. Neural crest cells and metastatic cancer cells share phenotypic similarities such as high motility and invasiveness and can follow the same migratory pathways. BMPs consist of dimers the chains of which are connected by disulfide bonds and this dimerization is a prerequisite for bone induction. BMPs are both active as homodimer and heterodimer molecules.

**Figure 4:** BMPs can bind to a type II specific receptor present on the cell membrane and recruit a type I receptor, forming a complex. These receptors are transmembrane serine/threonine kinase proteins that self-phosphorylate after formation of the BMP-receptor II-receptor I complex and acquire the ability to phosphorylate Smad proteins, a family of TGFβ transducers.

One member of the BMP family, the 35-kDa homodimeric protein BMP7, appears to be a prerequisite for induction of condensation and epithelializa­tion of metanephric mesenchyme in the kidney. Furthermore, knockout studies have shown that BMP7 (and BMP4) are essential for early morphogenesis of the eye. BMP7 knockout mice revealed deficient ocular growth, resulting in epithelial development disturbances, e.g. absence of the lens and abnormal retinal organization, with disorganization of the pigment layer of the retina. BMP7 also plays a role in various pathological conditions (inflammation, diabetic retinopathy, fibrosis). In mesangial cells of the kidney BMP7 counteracted TGFβ induced fibrosis, reversing the process of chronic renal injury and maintaining an epithelial phenotype. Moreover, BMP7 experimental therapy showed to halt progression and reverse the effects of chronic progressive kidney disease. BMP7 counteracts the increased expression of several extracellular matrix (EMC) proteins and CTGF (connective tissue growth factor).
One member of the family of BMP antagonists, differential screening-selected gene aberrative in neuroblastoma (DAN, also known as NO3) encodes a secreted protein in the cysteine knot super family. Nakamura et al. found that endogenous DAN mRNA was upregulated during retinoic acid-induced neuroblastoma differentiation, indicating that DAN might be involved in neuronal differentiation. During some stages of embryogenesis DAN has been found in the eye. Biochemical analyses have demonstrated that DAN bound directly to BMP2 and interfered with BMP4 activity. Over-expression of DAN in transformed cell lines suppressed the transformed phenotype and reduced growth rate. In animal models, DAN exhibited BMP antagonist activity and Wnt inhibition. Expression of DAN was significantly reduced in malignant fibroblast cells and similarly over expression of DAN inhibited proliferation of these cells.

Angiogenesis and vasculogenic mimicry

Angiogenesis (physiologic) or neovascularization (pathologic) is defined as the formation of blood vessels from pre-existing vessels. It extends and remodels the primitive vasculature, giving rise to a complex vascular network. Angiogenesis is a multi-step process, which is regulated by soluble factors and cell matrix interactions. Activation of endothelial cells by angiogenic stimuli derived from non-endothelial cells resulted in expression of proteolytic enzymes and in site-directed proteolytic activity. The extracellular matrix components surrounding the endothelial cells are degraded locally by proteases, allowing for chemotactic migration of endothelial cells toward angiogenic stimuli. Endothelial cells thereupon proliferate and form a new vessel with a lumen. Adjacent sprouts anastomose and form loops, which become perfused with circulating blood. In growing tumors it became apparent that the driving force triggering the formation of a vascular network is oxygen tension. If a tumor reaches the critical size of > 2mm the process of diffusion is no longer sufficient to cover all tissue and there will be a decrease in oxygen level. Under conditions of hypoxia functional HIF-1 (hypoxia-inducible factor) accumulates in the cell, which in turn drives the expression of a number of genes whose products encourage angiogenesis. Prominent among these is VEGF-A. Other angiogenic factors may also play a role in the angiogenic process of uveal melanoma. High expression of SPARC (secreted protein acidic and rich in cysteine), Cyr (cysteine rich) 61 and TF (tissue factor) in uveal melanoma is correlated with blood vessel rich areas of the tumor and an aggressive phenotype. TSP2 (thrombospondin), an anti-angiogenic protein, was associated with smaller areas of blood vessels. Endoglin, a transmembrane regulatory receptor for TGFβ, has been suggested to be a specific marker for angiogenesis in uveal melanoma.

The phenomenon of angiogenic switch is an important step in tumor progression. The body purposefully denies its cells the ability to readily induce angiogenesis. By doing so, the body erects yet another impediment to block the development of large tumors. The angiogenic
switch is the moment when the tumor successfully breaks this defensive barrier and cancer cells get the ability to induce blood vessel growth. Part of these tumor blood vessels is leaky and immature because the pericytes and smooth muscle cells are usually poorly recruited to the tumors.

Lymphatic vessels are also part of the vascular circulatory system. The lymphatic system is made up of an extensive network of capillaries, collecting vessels and ducts that permeate most of the organs. Unlike the blood vasculature, which forms a continuous loop, the lymphatic system is an open ended, one-way transit system. The vessels collect extravasated protein-rich fluid and lymphocytes that are transferred from lymphatic capillaries to the collecting lymphatic vessels and ultimately into venous circulation. The lymphatic vessels form a part of the immune system by continuously transporting the white blood cells within the lymphoid organs (spleen, tonsils lymph nodes etc.) and bone marrow. Abnormal function of lymphatic vessels is implicated in conditions such as lymphedema, inflammation and immune disease. Perhaps most importantly, the lymphatic vessels are involved in tumor metastasis. Lymphangiogenesis is not present in the normal eye or in uveal melanoma.

Vasculogenic mimicry describes the formation of perfusion pathways in tumors by highly invasive, genetically deregulated tumor cells. It is termed vasculogenic because, although these pathways do not form from pre-existing vessels, they distribute plasma and may contain red blood cells. It is termed mimicry because the pathways are no real blood vessels, but vascular function is mimicked. In vasculogenic mimicry of the patterned matrix type highly invasive tumor cells form looping patterns rich in extracellular matrix (ECM) in vitro. Vasculogenic mimicry patterns are composed of laminin, collagen IV and VI, fibronectin and glycosaminoglycans. There is a strong association between the histological proof of vasculogenic mimicry patterns in primary uveal melanoma and subsequent death by metastasis.

VEGF family of growth factors and VEGF receptors

The vascular endothelial growth factor (VEGF) family consists of 7 members (VEGF-A to E and placenta growth factor 1 and 2 (PIGF-1 and-2)). Their expression pattern is correlated with pathological and physiological angiogenesis. The VEGF family factors mediate signals by activating VEGF-receptor (VEGFR) including VEGF-R1, -2 and –3. Five isoforms of VEGF-A are generated from the human VEGF-A gene by alternative splicing (VEGF-A 121, VEGF-A 145, VEGF-A 165, VEGF-A 189 and VEGF-A 205). VEGF-A is expressed by many cell types throughout the body and acts as a paracrine factor on endothelial cells and non-endothelial cells such as tumor cells. At sites of apparent angiogenesis VEGF-A levels are increased in cells surrounding sprouting tissue. In adults VEGF-A expression is mostly related to conditions of pathological angiogenesis as indicated
by increased VEGF-A expression like in highly vascularized tumors or ocular diseases e.g. diabetic retinopathy. Consistent with this the expression of VEGF-A is potentiated in response to hypoxia and by activated oncogenes as well as a variety of cytokines. Protein levels of VEGF-A in the aqueous humor of eyes with uveal melanoma are correlated with patient survival, basal tumor diameter and tumor height. One report suggests that uveal melanomas express VEGF-A. In most ciliochoroidal melanomas VEGF-immunoreactivity is correlated with necrosis, but not with the occurrence of systemic metastasis or tumor angiogenesis.

VEGF-B is most closely related to VEGF-A. VEGF-B is, according to expression and gene targeting studies, supposed to play a role in vascularization of heart and skeletal muscles. VEGF-B is likely to act in a paracrine fashion as its receptor is almost exclusively located on endothelial cells. Several studies show that VEGF-B is present in malignant tumors and therefore may induce tumor angiogenesis.

VEGF-C stimulates the migration of endothelial cells and increases vascular permeability and endothelial cell proliferation, but at higher concentrations than VEGF-A. Unlike VEGF-A, the expression of VEGF-C does not appear to be regulated by hypoxia, but is increased in response to proinflammatory cytokines. VEGF-C can regulate physiological and pathological blood vessel growth in vivo. Furthermore, VEGF-C has been shown to regulate the growth of lymphatic vessels. The angiogenic versus lymphangiogenic responses to VEGF-C may depend on the degree of proteolytic processing of its precursor and on the expression of its receptors in the blood versus lymphatic endothelial cells of the target tissue. Signals for endothelial cells are probably mediated through VEGFR-2 (KDR) in

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**Figure 5:** Binding of VEGFs to the various receptors is depicted.
blood vascular endothelial cells and via VEGFR-3 (Flt 4) in lymphatic endothelial cells. VEGF-C furthermore acts synergistic with VEGF-A during the induction of angiogenesis. In a molecular profiling study, VEGF-C was significantly upregulated (30-fold) in highly versus poorly invasive uveal melanoma cells. Clarijs et al. showed that VEGF-C was not able to induce lymphangiogenesis in the normal eye and in uveal melanoma. VEGF-C has been found to induce the formation of new blood vessels, but only in early development or in certain pathological settings such as tumorigenesis.

VEGF-D is the most recently discovered member of the family and shares 61% sequence identity with VEGF-C. These two growth factors bind to the same receptors on human endothelial cells. VEGF-D has been shown to be able to stimulate the proliferation of endothelial cells. Stacker et al. showed that VEGF-D can induce lymphangiogenesis and thereby allow the access of tumor cells to the lymphatic network.

Animal models related to uveal melanoma development, progression and metastasis

Prospective and retrospective data of clinical studies are important factors in uveal melanoma research. Registries of ocular pathology have also played an essential role in acquiring knowledge of this malignant neoplasm. One of the main limitations of research is the low incidence of this tumor, making it difficult to achieve a representative number of cases. Moreover, with the increasing use of conservative treatments in the last few decades the number of enucleation specimens for pathology has decreased. For these reasons the development and use of experimental models is necessary. Many attempts have been made to develop a suitable animal model to study more effectively the etiology, pathogenesis, diagnosis, metastasis and therapy of uveal melanoma. Uveal melanoma may spontaneously occur in some animals, specifically mammals. The different histological features, metastatic behavior and unpredictable nature of occurrence of these uncommon spontaneous tumors in comparison to human ocular melanoma diminish their suitability as a model. Several models have been developed to induce an intraocular tumor chemically or by radiation of animals. Some of these induced tumors might resemble human uveal melanoma, although the majority originates from retinal pigment epithelium. Various viruses have been used to biologically induce uveal proliferations, disadvantages is the high mortality among infected animals. Injected animals often develop disseminated viral infections and secondary tumors induced by virus shed from primary intraocular melanoma rather than metastases. Inoculation of tissue culture of hamster-, murine- or human melanoma cells into animal eyes have the advantage that the inoculation site and size may be controlled. Unfortunately, immune suppression occurs in some models. Transgenic murine models have been developed, using the promoter region of tyrosinase gene to target expression of oncogenes in melanine producing cells. The tyrosinase gene was chosen because it drives the expression of downstream genes in melanocytes. Human uveal melanoma cell lines are also used to
induce orthotopical tumors and distant metastasis \[171\]. Cells can be injected into the anterior chamber of the eye of nude mice \[172\]. Some cell lines will develop distant metastasis after enucleation of the orthotopically-induced tumor \[154, 165, 173\]. Disadvantages of these models are problems with monitoring tumor growth during the experiment and localization of the metastasis.

In conclusion, the ideal animal model of uveal melanoma should meet a number of conditions \[174\]:

- There should be structural similarities between the animal eye and the human eye.
- The cellular physiological behavior and reaction to drug administration should be similar for animals and humans.
- The etiology and evolution of the disease should be comparable to the human pattern.
- The rate of tumor outgrowth should be as high as possible (near 100\%) in order to minimize the use of animals.
- Tumor growth should be follow-able directly and quantitatively for adequate follow-up of the disease and the effect of the treatments administered.

Several animal models have been used to study primary and metastatic uveal melanoma as well as treatments for these tumors and ways to prevent metastasis.

In the study of Clark et al. \[175\], the effect of the angiostatic agent anecortave acetate was studied in an animal model of a murine uveal melanoma cell line injected into the anterior chamber of the eye. Restricted growth was observed after topical treatment. Interferon-alpha-2b was tested for its effect on metastatic disease of melanoma cells inoculated into the posterior chamber of the eye \[176\]. It resulted in decreased development of metastasis.

Ma et al. \[177\] developed a human uveal melanoma model in nude mice with viral overexpression of PAI-1, which resulted in a reduction of liver metastasis. They also studied the relation of epidermal growth factor receptor (EGFR) expression and metastasis of uveal melanoma in nude mice model.

### Outline of the thesis

The studies described in this thesis are an attempt to increase the knowledge with respect to biological mechanisms in uveal melanoma in vitro, in vivo and in patients. This knowledge might be used to consider new treatment modalities for uveal melanoma and its metastatic disease. A new animal model for uveal melanoma described in **Chapter 2** provides a method to follow tumors cells in a living animal. This xenograft model, based on real time bioluminescent imaging of luciferase-expressing human melanoma cells, allows for cell tracking and non-invasive monitoring of tumor growth, metastasis and therapeutic response.

The role of BMP7 in tumor progression was studied, using a human uveal melanoma cell
line and transfecting this cell line with the gene of interest, BMP7 (Chapter 3). In the study described in Chapter 4 we investigated the tumor suppressor gene DAN, an antagonist of the BMP pathway, in enucleation specimens of uveal melanoma patients. Angiogenesis as an important process in melanoma development is described in Chapters 5 & 6. Chapter 5 describes the VEGF-family factors and their profile in vitro, in vivo and in patients at a molecular level. The study in Chapter 6 presents the correlation of tumor parameters with the presence of angiogenic factor VEGF-A in the aqueous humor of patients. In the Discussion (Chapter 7) implications of the findings presented in this thesis on the understanding of the biological mechanisms are discussed in detail, with special emphasis on their relevance to uveal melanoma tumor growth and metastatic behavior.

References


34. Prescher G, Bornfeld N, Becher R. Two subclones in a case of uveal melanoma. Relevance of
monosomy 3 and multiplication of chromosome 8q. Cancer Genet Cytogenet. 1994;77:144-146.


107. Hsu DR, Economides AN, Wang X, Eimon PM, Harland RM. The Xenopus dorsalizing factor Gremlin


162. Folberg R, Barron BC, Reeves R.D. Primary melanocytic lesions of the rabbit choroid following topical application of 7,12-dimethylbenz[a]-anthracene: preliminary observations. *J Toxicol Cutan Ocul*


