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Genetics of Uveal Melanoma

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1. Introduction

Uveal melanoma is the most common type of primary eye cancer in adults, affecting 0.7/100,000 of the Western population yearly (Egan et al., 1988). The melanoma originates from neural crest derived melanocytes of the uvea (choroid, ciliary body and iris) and despite enucleation or conservative treatment half of patients die of, most often late appearing, metastatic disease (15-year survival: 53%) (Diener-West et al., 1992; Gamel et al., 1993; Kujala et al., 2003). To detect these (micro) metastasising cells in an early phase is one of the main challenges in the (uveal melanoma) oncology field and a prerequisite for proper patient selection in future therapeutic interventions. Several clinical, histological and genetic markers have been identified to predict poor prognosis in uveal melanoma patients. Genetic markers as chromosome 3 loss or the expression of a specific set of genes have proven to be far out the most significant prognostic markers. This will not only facilitate diagnosis and prediction of prognosis but will also assist in selecting patients for adjuvant therapy and the monitoring of circulating tumour cells. Alternatively, some of the tumour markers as *GNAQ*/*GNA*11, or *BAP*1 may serve as targets for new types of intervention tackling that specific pathway.
In this chapter, the most recent cytogenetic and molecular genetic approaches will be discussed along with the most important findings and their value for current and future management of patients with uveal melanoma.

2. Clinical aspects of uveal melanoma

2.1 Diagnosis

The diagnosis of uveal melanoma is based on ophthalmic examination using ancillary tests (ultrasonography, transillumination, optical coherence tomography) and occasionally fluorescein angiography, computed tomography and magnetic resonance imaging) and photography for follow-up. (Figure 1). Approximately 30% of patients have no symptoms at time of diagnosis (Damato 2010). Upon diagnosis of the primary tumour, patients are screened for metastases by liver enzyme tests and liver ultrasound and at that moment, less than 2% patients have detectable metastases (Shields, J. A. et al., 1991).
The primary uveal melanoma is located either in the choroid (72%), in the ciliary body (23%) or in the iris (5%). Choroidal melanomas usually present as a discoid, dome-shaped or mushroom-shaped subretinal mass, whereas ciliary-body melanomas regularly present as sessile or dome-shaped lesions. Iris melanomas may also present as dome-shaped

lesions or diffuse lesions and are the least common type of uveal melanoma. Iris melanomas tend to present at a smaller size, probably because pigmented lesions of the iris are usually visible to the patient at an early stage, which adds to a favourable prognosis. Iris melanomas may cause blockage of the drainage angle and lead to secondary elevation of intraocular pressure (Shields, C. L. et al., 2001). In contrast to iris melanomas, melanomas located in the ciliary body are associated with a high metastatic potential (Schmittel et al., 2004).

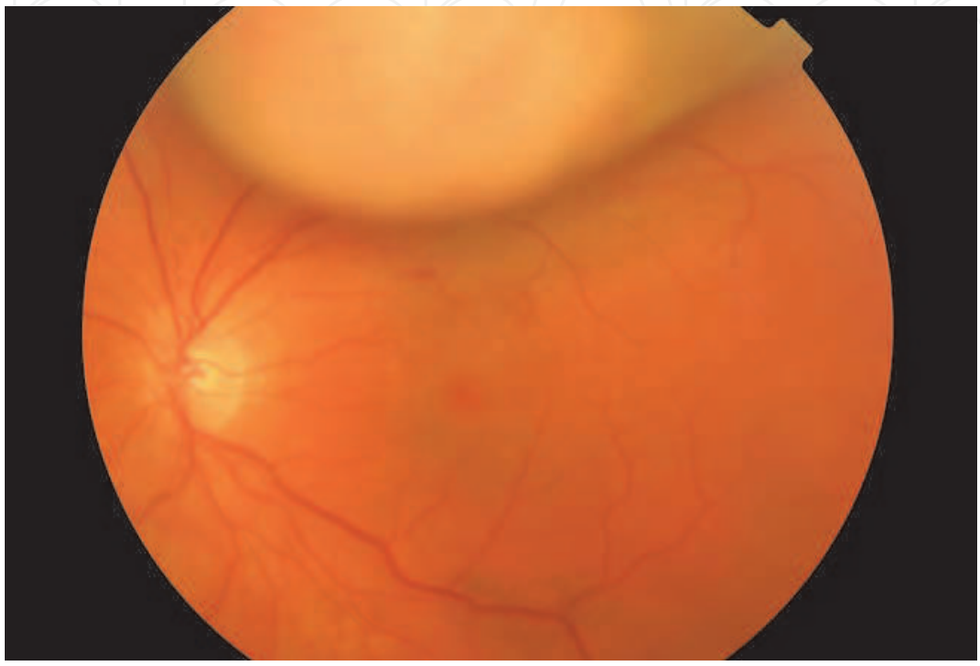

Fig. 1. Fundus photography showing a superiorly located uveal melanoma in the left eye.

If enucleation or biopsy is performed, the diagnosis is confirmed by histopathological examination (Figure 2). Melanomas consist of spindle, epitheloid cells or a mix of both cell types, and haematoxylin and eosin (H&E) staining is used to differentiate between these cell types. Periodic-acid Schiff (PAS) staining helps to identify microvascular patterns (three closed loops located back to back). Additional melanocytic markers that can be used in immunohistochemistry are S-100 or HMB-45.

Fig. 2. Haematoxylin and eosin (H&E) staining of 2 tumour sections showing choroidal melanoma (left) and ciliary body melanoma (right).

2.2 Predisposing factors

Men and women are equally affected by uveal melanoma and most patients are 60 years of age and older. Certain phenotypes have been described, predisposing to uveal melanoma. Caucasian race for instance, is the most important known to date: uveal melanoma is approximately 150 times more common in Caucasians than in Africans (Margo et al., 1998; Singh et al., 2005). Furthermore, blue or gray eyes as well as fair skin type and inability to tan have been suggested to predispose to uveal melanoma (Gallagher et al., 1985; Schmidt-Pokrzywniak et al., 2009; Tucker et al., 1985). Although these facts may point towards a possible role of UV-radiation in the development of uveal melanoma, current evidence regarding UV-radiation is still inconclusive (Li et al., 2000; Manning et al., 2004; Marshall et al., 2006; Singh et al., 2004; Vajdic et al., 2002). There is however a tendency for iris melanomas to occur in the lower half of the iris, which has been explained by the increased sunlight exposure of this area (Shields, J.A. & Shields 2007).

Specific conditions as ocular and oculodermal melanocytosis (Nevus of Ota) (Gonder et al., 1982; Singh et al., 1998), neurofibromatosis type I (Wiznia et al., 1978), dysplastic nevus syndrome (Albert et al., 1985) are all associated with an increased incidence of uveal melanoma. Although uveal melanoma is rarely hereditary, several familial cancer syndromes have been reported: xeroderma pigmentosa, Li-Fraumeni syndrome and familial breast and ovarian cancer (Travis et al., 1996; Wooster et al., 1994). The low incidence of familial uveal melanoma cases limits approaches such as linkage analysis for the identification of susceptibility genes (Singh et al., 1996; Triozzi et al., 2008).

2.3 Clinical prognostic factors

The predictive value of classic prognostic parameters such as age, tumour size, tumour location, histological cell type and presence of vascular loops has been analysed in several retrospective studies (Coleman et al., 1993; Mooy & De Jong 1996). These parameters were complemented by the more recent identification of other histological (tumour-infiltrating lymphocytes, protein biomarkers) and genetic parameters (chromosomal aberrations, expression profiling) (Kujala et al., 2003; Naus et al., 2002; Patel, B. C. et al., 1998; Petrausch et al., 2008; Sisley et al., 2006; Tschentscher et al., 2003; van den Bosch et al., 2010; van Gils et al., 2008b). Tumour size (largest tumour diameter) is the most important clinical prognostic parameter and because of its ease of determination with ultrasonography, most often used for therapy planning. The 5-year mortality rate in patients with tumours below 10 mm in diameter is approximately 15% and increases to 53% for tumours larger than 15 mm in diameter (Gamel et al., 1993). Tumours located in the ciliary body correlate with progressive disease (Schmittel et al., 2004). The same holds true for tumours that show scleral invasion, optic nerve invasion, or extraocular extension (Damato 2010; McLean et al., 2004).

Histological presence of epitheloid cells and closed vascular patterns are also strongly associated with early death from uveal melanoma (Folberg et al., 1993; Maniotis et al., 1999; Seddon et al., 1983). These histological prognostic factors as well as genetic factors are less frequently used for primary therapy planning as tumour tissue is required for the pathological and genetic assessment of risk factors. In most cases, enucleation enables research on tumour tissue from relatively large-sized tumours. More frequent use of in-vivo biopsy prior to therapy may help assessing genetic risk factors, also in smaller tumours that may be treated conservatively. Several groups have already proven fine-needle biopsy to be a reliable technique yielding sufficient tumour tissue for cytogenetic analysis (Midena et al., 2006; Naus et al., 2002; Shields, C. L. et al., 2011).

Clinical, histological, and cytogenetic factors can be used to identify patients with high risk of metastases from uveal melanoma (Eskelin et al., 2000). As micrometastases are thought to arise early in the disease and precede clinically detectable macro metastases, present prognostic factors may thus be used to identify patients with micrometastatic disease.

2.4 Metastasis

Uveal melanomas metastasise almost exclusively by haematogenous route, and about 90% of patients with metastatic disease have hepatic metastases (Bedikian et al., 1995; Gragoudas et al., 1991). Other, less frequent sites for metastases include lung, skin, bone and brain (Collaborative Ocular Melanoma Study 2001; Diener-West et al., 2004; Gragoudas 2006; Landreville et al., 2008). Involvement of regional lymph nodes is rare and is attributed to the absence of draining lymphatics of the eye. Extraocular extension of tumour tissue though, may result in occasional metastatic involvement of lymph nodes.

The 15-year disease specific survival rates for patients with uveal melanoma is: 53% (Gamel et al., 1993). Shields et al (Shields, C. L. et al., 2011) recently reported a 3-year peak mortality of 24%. This could indicate a possible state of tumour dormancy or latency where circulating tumour cells remain silent and undetectable for the first 2 years after diagnosis (Klein 2011). Metastatic disease only rarely responds to treatment, and is usually fatal within 2-9 months after onset of symptoms (Diener-West et al., 2005; Eskelin et al., 2003). If the liver is involved, survival is most of the time shorter than 3 months. Treatment by systemic or intra-hepatic chemotherapy or partial hepatectomy rarely prolongs life (Augsburger et al., 2009). This highlights the urgent need for new and more effective therapies.

2.5 Fine needle biopsies and tumour heterogeneity

In previous research we have substantiated that specific regions on chromosome 1 and 3 are important in the aetiology of uveal melanoma (Kilic et al., 2005). Both our genetic and expression profiling studies point towards certain areas of the genome, that are important in tumour development and progression (van Gils et al., 2008a; van Gils et al., 2008b). As most cytogenetic and molecular genetic studies up till now involve patient samples from large tumours treated by enucleation, no specific knowledge is currently available for patients who receive conservative treatment such as stereotactic radiation therapy. Even though the melanomas treated by stereotactic radiotherapy are smaller than those treated by enucleation, still 25% of these tumours metastasise (van den Bosch T, manuscript in preparation). This implies that also smaller-sized tumours have the typical chromosomal aberrations required for dissemination of the disease. Cytogenetic and molecular genetic analyses of smaller tumours will most likely give more insight into tumour evolution and may enable identification of less complex chromosomal aberrations in uveal melanoma. In-vivo biopsy will be crucial for gaining tissue of small-sized tumours.

Previous results, with Fluorescent in situ hybridisation (McGill et al.,) (FISH) on Fine-needle aspiration biopsies (FNAB) acquired tumour tissue, showed that adequate FNAB material can be collected in a reliable and safe way for FISH analysis (Naus et al., 2002). The risk of local metastasis due to biopsy taking was found not to be increased with the FNAB technique (Char et al., 1996), and even a lower risk is reported if a transvitreal route is chosen for FNAB (Glasgow et al., 1988; Karcioglu et al., 1985). Tissue structure is also recognisable next to the single cells that have been aspirated with FNAB. Bechrakis et al. combined a vitrectomy with a biopsy and showed that in 97% of the biopsies histological

diagnosis was possible (Bechrakis et al., 2002). So there is a growing preference using this technique, especially since it is a more controllable approach and yields more material, on which in addition to cytogenetic and molecular genetic techniques histological examination will be possible.
Heterogeneity of monosomy 3 (complete loss of chromosome 3) in uveal melanoma has been studied by FISH analysis on paraffin embedded tumour material, and on single-cell suspensions of fresh tumor tissue and showed that a difference in percentage of monosomy 3 may be present in some cases. However, our earlier results, where FISH on FNAB and tumour samples were compared, showed this to lead to misclassification in less than 1% (Naus et al., 2002). Tumour heterogeneity was further investigated and it was concluded that analysis of tumour biopsies in uveal melanoma gives an accurate prediction of the high-risk characteristics (Mensink et al., 2009b). In a more thorough study, we showed that hyperdiploidy (60-70 chromosomes) often resulted in copy number loss of chromosome 3, with loss of heterozygosity of one allele (Mensink manuscript submitted).

2.6 Therapy

Until the late eighties, the only treatment available was enucleation of the affected eye. Nowadays, conservative treatment protocols such as brachytherapy, thermotherapy, or radiation therapy may be used to treat small and medium-sized tumours with conservation of the eye additionally. Large-sized melanomas however are preferably still treated by enucleation (Shields, J. A. et al., 1996). The survival rate of patients with metastatic disease remains extremely poor as none of the current therapies proves to be effective. Several different cytotoxic agents such as dacarbazine have been administered alone, or in combination with other chemotherapeutic drugs or interferon-alfa-2b to high-risk patients after primary therapy. These regimens however, have not led to improved outcomes for these patients yet (Triozzi et al., 2008).
Despite improvements in treatment protocols for primary tumour and metastatic disease, and despite the fact that hardly any of the patients have clinical detectable metastasis at presentation, still half of all patients die of metastatic disease (Kujala et al., 2003).
Unfortunately no effective therapy exists for the treatment of metastatic disease at this moment, but new protocols involving combinations of chemotherapy and immunotherapy have been initiated recently. Systemic therapy may be more effective if administered early after diagnosis treating micrometastatic rather than macrometastatic disease. In the latter case, multiple mechanisms of resistance against systemic interventions may be present (Triozzi et al., 2008). With this in mind a new adjuvant immunotherapy protocol has been developed, where clinical, histological, and cytogenetic factors are used to identify high-risk uveal melanoma patients and treat these by immunization with their own trained dendritic cells to prevent future metastatic disease. This multicenter trial is performed by the ROMS in collaboration with Radboud University Nijmegen.

3. Molecular aspects of uveal melanoma

Cancer development is often associated with genomic instability and acquisition of genomic heterogeneity (Bayani et al., 2007), generating both clonal and non-clonal tumour cell populations (Katona et al., 2007; Ye et al., 2007). Several mutations in the cell cycle can lead to aneuploidy: during mitosis, spindle checkpoint processes such as chromosome attachment to the spindle and chromosome segregation are vulnerable to changes leading to single point

mutations or even gross chromosomal rearrangements (Kops et al., 2005; Olaharski et al., 2006). There is delicate balance between a possible benefit from the accumulation of genetic and epigenetic alterations and a lethal genetically unstable state of the cells (Nguyen & Ravid 2006). Polyploidy is also well known in cancer and it tends to occur in tumours with a more aggressive phenotype (Castedo et al., 2006; Kaneko & Knudson 2000).
Research is focusing on finding pathways involved in carcinogenesis, thereby trying to understand tumour onset and early development and transition to metastatic disease. Highly invasive tumours are compared with poorly invasive ones, primary tumours with its metastases, and therapy-resistant tumours with responsive ones in order to search for differentially expressed genes and specific chromosomal regions or genes involved in these processes.

3.1 Cytogenetic and molecular genetic techniques

A wide variety of cytogenetic and molecular genetic techniques are available and others still in development. Short term cultured uveal melanoma specimens are very suitable for classic cytogenetic analysis and spectral karyotyping (SKY), and these generally display display a relatively simple karyotype with recurrent chromosomal anomalies. (Figure 3)
Fluorescent in situ hybridisation, comparative genomic hybridisation (CGH) and quantitative PCR techniques can be applied to fresh or frozen tissues, cell lines, and archival formalin-fixed paraffin-embedded samples.

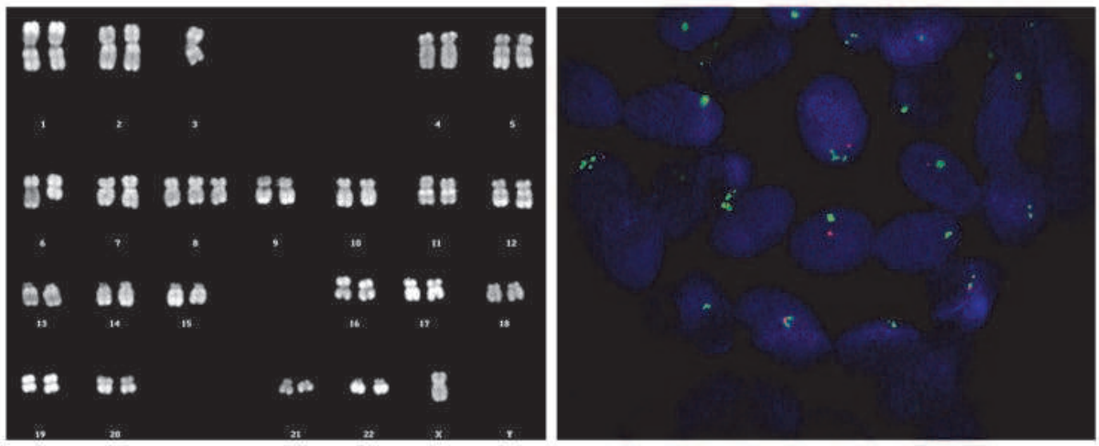

Fig. 3. Karyogram showing loss of chromosome 3, isodisomy of 6p, and gain of chromosome 8 (left). FISH: nuclei showing one signal for chromosome 3p (red) and centromere 3 (green) (right).

Currently microarray based CGH, SNP analysis and gene expression analysis are the most frequently applied techniques. A drawback of array-based approaches is that the analysed signal represents the average value of all cells in the tumour sample, requiring a high signal-to-noise ratio to quantitatively and reliably detect low-level DNA copy number changes on individual array elements (Albertson et al., 2003). The great advantage is that expression and copy number information on thousands of gene and chromosome locations can be obtained from a single mRNA or DNA sample in just one experiment.
Recently Next Generation Sequencing (NGS) has been applied on primary uveal melanoma samples resulting in the detection of mutations, showing single or multiple base pair changes.

A brief summary of the current findings is outlined below (The technical aspects of these techniques have been reviewed by us (Mensink et al., 2009a) and others (Harbour 2009) recently.

3.2 Chromosomal anomalies as prognostic markers

Specific chromosomal anomalies, as deletion of chromosome 1p, monosomy of chromosome 3 or gain of chromosome 8q, strongly correlate with decreased survival in uveal melanoma.
Monosomy of chromosome 3 is the most frequently found non-random chromosomal aberration in uveal melanoma and is predominantly found in metastasising tumours (Prescher et al., 1996). In univariate analysis, monosomy 3 was the most significant predictor ($p < 0.0001$) of poor prognosis in uveal melanoma, followed by tumour location and diameter (Prescher et al., 1996). It is considered to be a primary event, because it is seen in combination with all other chromosomal aberrations in uveal melanoma such as loss of chromosome 1p, gain of 6p and gain of 8q (Prescher et al., 1995). In the majority of tumours with chromosome 3 losses there is complete monosomy, although occasionally isodisomy is acquired (Aalto et al., 2001; Scholes et al., 2001; White, V. A. et al., 1998). Rarely, melanomas with partial aberrations on chromosome 3 or translocations have been described, making it difficult to map putative tumour suppressor genes. Loss of heterozygosity studies demonstrate common regions of allelic loss located on 3p25 and on the long arm spanning from 3q24 to 3q26 (Onken et al., 2007; Parrella et al., 2003).
Concomitant loss of chromosomes 1p and 3 has a stronger correlation with metastasising disease than either one of them separately (Figure 4) (Kilic et al., 2005). The common deleted region on chromosome 1 ranges from 1p34.3 to 1p36.2 (Aalto et al., 2001; Hausler et al., 2005; Hughes et al., 2005).

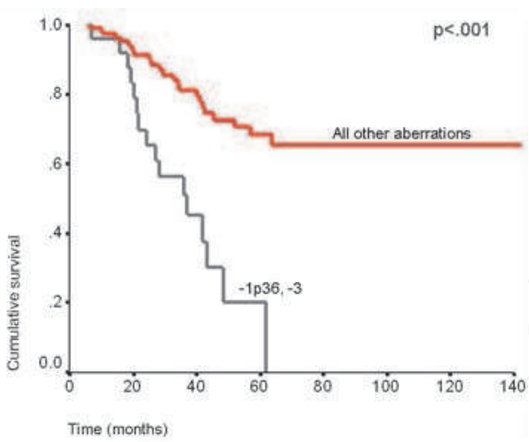

Fig. 4. Kaplan-Meier survival curve of UM patients with tumours showing loss of chromosome 1p36 and chromosome 3. Figure adapted from (Kilic et al., 2005).

The association with chromosome 8q gain was slightly less significant than for monosomy 3, but a strong inverse correlation ($p < 0.0001$) of dosage effect of additional copies of 8q on survival was observed (Sisley et al., 1997). Gain of chromosome 8, or acquisition of an isochromosome 8q, is suggested to be a secondary event in uveal melanoma, because variable copy numbers of 8q can be present in one tumour (Horsman & White 1993; Prescher et al., 1994). It occurs frequently in tumours that have lost one copy of chromosome

3 and it is an independent prognostic factor of progressive disease (Patel, K. A. et al., 2001; Sisley et al., 1997; White, V. A. et al., 1998). The shortest region of overlapping gain spans from 8q24.1 to 8qter (Hughes et al., 2005; Sisley et al., 2006).

In a series of large posterior uveal melanomas, presence of a chromosome 6p abnormality was predictive of good outcome (White, V. A. et al., 1998). These tumours with gain of chromosome 6p have been proposed to represent a separate group of uveal melanoma with an alternative genetic pathway in carcinogenesis, because gain of 6p is frequently found in tumours with disomy 3 (Ehlers et al., 2008; Hoglund et al., 2004; Landreville et al., 2008; Sisley et al., 1997).

Abnormalities of other chromosomes have also been detected in uveal melanoma. However, they often lead to contradictory results regarding the prognostic impact. Chromosome 18q22 has been suggested to play a prognostic role(White, J. S. et al., 2006), but this could not be confirmed by other groups (Mensink et al., 2008). Chromosome 9p21(Scholes et al., 2001) and chromosome 16q (Vajdic et al., 2003) have been described to be important in uveal melanoma as well.

SNP arrays can simultaneously be used to define copy number changes in tumours from signal intensities reflecting the amount of hybridised DNA (Bignell et al., 2004), and for determining and mapping of chromosomal regions with loss of heterozygosity. The great advantage is that information on thousands of locations or genes can be obtained in a single experiment with a high resolution. With SNP-array we and others were able to confirm the frequently found alterations by FISH on chromosomes 1p, 3, 6p, 6q, 8p and 8q (Figure 5). Other chromosomal alterations were found by FISH: +7, -9p, -10 or +10, -11q23-q25 (van den Bosch et al., 2010).

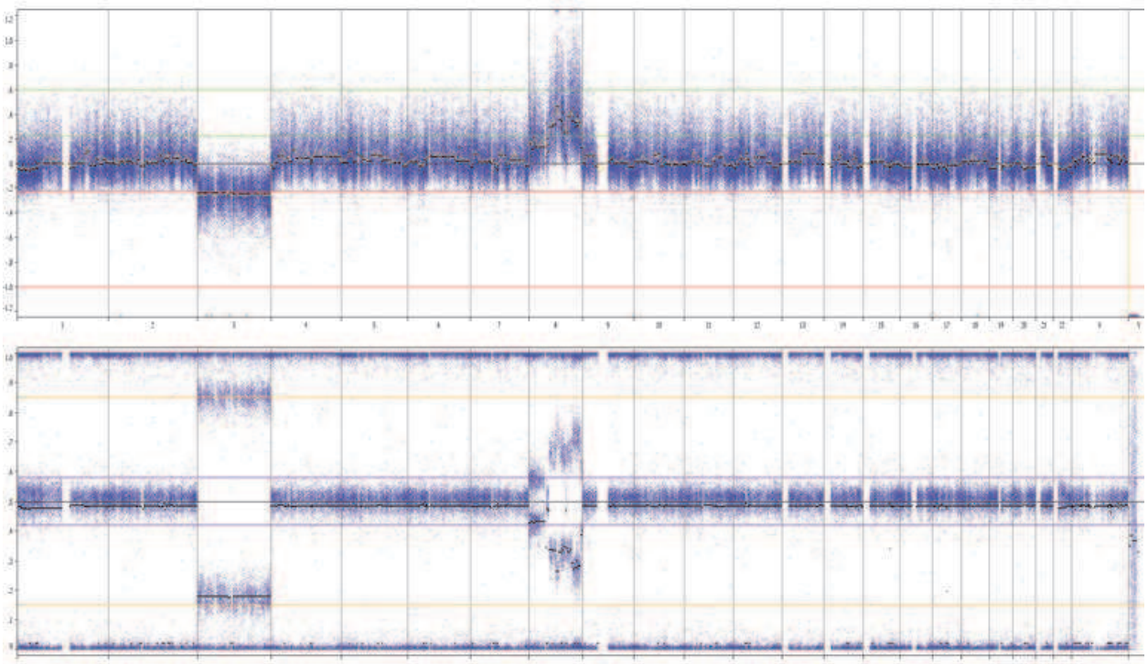

Fig. 5. SNP-array results of a patient with uveal melanoma, showing monosomy 3 and gain of chromosome 8q (LogR ratio in upper panel). Lower panel represents B- allele frequency showing allelic imbalance of chromosome 3 and chromosome 8.

Partial losses of chromosome 3 are very rare and therefore also rarely reported in the available literature (Parrella et al., 2003; Trolet et al., 2009; Tschentscher et al., 2001). In nearly all cases, complete loss of one copy of chromosome 3 is found, even in the smaller-sized melanomas that had been enucleated. With the recent high-resolution SNP-array's, no specific small regions of loss on chromosome 3 have been found.
For the fact that most uveal melanomas have complete loss of chromosome 3, either FISH, q-PCR or SNP-array may be used for analysing chromosome status in patients with uveal melanoma. There is thus no advantage of either SNP-array or FISH for only detecting chromosome 3 alterations in uveal melanoma. SNP-array though, allows for testing of multiple loci on different chromosomes compared to just one or two loci per chromosome with FISH or q-PCR. SNP-array is a fast measure for genome-wide assessment of chromosomal aberrations whereas FISH takes more time as multiple experiments would be necessary to assess multiple chromosomes or loci. There is one major difference between SNP-array and FISH, being that SNP-array enables assessment of heterozygosity, potentially revealing regions with loss of heterozygosity or allelic imbalances. This feature may provide us with information especially in disomic chromosome 3 cases where one allele is lost and the remaining one is copied by mistake (isodisomy) or uniparental disomic states. These processes may lead to loss of heterozygosity and analysis of these cases may help find an answer for patients with disomy 3 who still developed metastasis.

3.3 Gene-expression profiling

Based on expression data uveal melanomas can be divided in two classes. We and others showed these two classes to correspond remarkably well with the ability of the tumour to metastasize. When compared to clinico-pathologic or genetic prognostic markers, this classification on basis of a set of classifier genes is far-out superior (Petrausch et al., 2008; Tschentscher et al., 2003; Worley et al., 2007). In general the top classifier list revealed a global down-regulation of neural crest and melanocyte-specific genes and an up-regulation of epithelial genes in "metastatic" class II. These tumours exhibit epithelial features, such as cell morphology, and up-regulation of the E-cadherin pathway (Worley et al., 2007). RNA extraction from enucleated tumour or a fine needle biopsy is feasible and can be used for transcriptomic analysis on uveal melanoma samples, this service is in fact currently commercially available (Onken et al., 2010). Mentioned expression studies yielded similar sets of classifier genes however these classifier genes are merely markers of the underlying cause whereas genes involved in tumour progression and metastatic potential still have to be discovered. (van Gils et al., 2008b), If these are genes encoding cell surface markers, they could be a target for cell therapy aimed at an immunological response to eliminate tumour cells.

3.4 Next Generation Sequencing

The introduction of Next Generation Sequencing provides chromosomal mutational analysis up to the base-pair level. This sequencing technique therefore has the highest resolution possible and with the possibility for fast genome-wide testing in multiple tumour samples at once, is a very precise and reliable technique for mutational analysis. A limitation is the fact that preceding PCR amplification is required most of the time, which may introduce mutations due to the error rate of polymerase enzyme. The most recent genes involved in uveal melanoma however were found by sequence analysis, such as *BAP*1 and *GNAQ*. These genes are discussed in more detail further on.

3.5 Epigenetic regulation

Epigenetic mechanisms are known to alter genomes by other ways than direct changes in DNA sequence. For example, genes and promoters may have their functions silenced by methylation processes. In uveal melanoma, methylation of *CDKN2A* is present in 4 to 32% (Merbs & Sidransky 1999), *RASSF*1 in 13 to 70% (Maat et al., 2007), *RARB* up to 7% and *TIMP*3 in 9% (van der Velden et al., 2003; van Gils et al., 2008b). *RASEF* is targeted by loss of heterozygosity in combination with methylation in primary uveal melanoma; there is only low percentage methylation (Maat et al., 2008). *hTERT*, an important gene in carcinogenesis, is methylated in up to 52% of uveal melanoma, whereas *FHIT* and *APC* are never hypermethylated (Merhavi et al., 2007; Moulin et al., 2008; Zeschnigk et al., 2003). In none of these studies hypermethylation of a gene correlated with metastatic disease. When we looked for regions containing blocks of up or downregulated genes using LAP analysis specific regions with significantly low or high expression on the genome were apparent (van Gils et al., 2008b). These local over or underexpression could be the result of small deletions or amplifications. Alternatively epigenetic mechanisms as hyper or hypomethylation could be an explanation.

3.6 Genes involved in uveal melanoma

Deregulation of the RAS-RAF-MEK-ERK or mitogen-activated protein kinase (MAPK) pathway is common in many human malignancies (Inamdar et al., 2010). Mutations of specific members of these key molecular signaling pathways have been implicated in tumourigenesis of cutaneous melanoma. Many of these often well known oncogenes (e.g. *NRAS*, *BRAF*) or tumour suppressor genes (*PTEN*, *CDKN2A*) have also been analysed in uveal melanoma and occasionally mutations in these genes were found. Although no relation with development of metastatic disease was found, the presence of somatic mutations in these genes can provide a starting point for early detection of metastatic cells in blood or therapeutic intervention.

More promising are the recent findings of two frequently mutated members of the MAP-kinase pathway, *GNAQ* and *GNA*11, and the *BAP*1 gene.

3.6.1 The MAP-kinase pathway: GNAQ and GNA11

Recently Van Raamsdonk and coworkers demonstrated that approximately 80% of uveal melanomas carried activating mutations in either *GNAQ* or *GNA*11, turning these into oncogenes (Herlyn & Nathanson 2010; Van Raamsdonk et al., 2009; Van Raamsdonk et al., 2010). These genes belong to a subfamily of genes encoding for the G-protein α subunit involved in MAPK cell signaling. Mutations in the G-protein α stimulatory subunit of *GNAQ* were found in 46% of uveal melanoma cases, wheras mutations of *GNA*11 were found in 35% of uveal melanoma cases. Both of these genes showed to be mutually exclusive and were by their oncogenic conversion suggested to be the cause of constitutive MAP-kinase pathway activation. This in turn leads to cell proliferation even in the absence of extracellular stimuli.

Activating somatic mutations of *GNAQ* at codon 209 were found by Onken et al, in 31 of 58 (54%) posterior uveal melanomas, and in two of nine (22%) iris melanomas(Onken et al., 2008; Romano et al., 2011). Iris melanomas thus less often show *GNAQ* mutations, but can occasionally have mutant *BRAF* (Henriquez et al., 2007; Onken et al., 2008; Sisley et al., 2011; Van Raamsdonk et al., 2009). Conjunctival melanomas on the other hand often have *BRAF* involvement but do not frequently have *GNAQ* mutations(Dratviman-Storobinsky et al., 2010).

These specific *GNAQ*/*GNA*11 mutations are also found in 83% of blue naevi of the skin (Van Raamsdonk et al., 2009) and are present in all stages of progression (Sisley et al., 2011). These mutations therefore are thought to occur early in tumourigenesis, which is underlined by the fact they are not correlated with either molecular class or metastasis in general (Bauer et al., 2009; Onken et al., 2008).

3.6.2 The RAS-RAF-MEK-ERK pathway

Mutations in the RAS-RAF-MEK-ERK (MAPK) pathway are thought to be early or initiating events in tumorigenesis (Onken et al., 2008). In general, this pathway is activated by autocrine growth factor stimulation or by mutation of *BRAF* or *RAS* genes (Dhomen & Marais 2009; Fensterle 2006; Mercer & Pritchard 2003; Zuidervaart et al., 2005) resulting in excessive cell proliferation. A single substitution (p.V600E) appears to account for more than 90% of all *BRAF* mutations in cutaneous melanoma and this mutation is also frequently found in benign and premalignant nevi thereby suggesting these to be early events in tumourigenesis (Davies et al., 2002; Pollock et al., 2003). *BRAF* and *NRAS* are both activators of the MAPK pathway though mutations of these genes are very rare in uveal melanoma (Cohen et al., 2003; Kilic et al., 2004; Mooy et al., 1991; Saldanha et al., 2004).
Activation of the MAPK pathway appears to be a common event through *GNAQ*/ *GNA*11-mutation induced G-protein signaling and possibly also by activation of ERK, a downstream kinase of this pathway (Calipel et al., 2006; Weber et al., 2003).
It has been suggested that MAPK activation in uveal melanoma may also arise via crosstalk with the PI3K-PTEN-AKT pathway (Zuidervaart et al., 2005).

3.6.3 The PI3K-PTEN-AKT pathway

The tumour suppressor gene phosphatase and tensin homolog (*PTEN*), is involved in the PI3K pathway as negative regulator of AKT. Loss of function of *PTEN* by deletion or mutation, leads to activation of AKT and overexpression of the PI3K-PTEN-AKT pathway preventing apoptosis (Ehlers et al., 2008; Ibrahim & Haluska 2009). Inactivation of *PTEN* is reported in 15% of uveal melanoma cases and has been linked to an increase in aneuploidy but also poor clinical outcome (Abdel-Rahman et al., 2006; Ehlers et al., 2008). This may suggest a role in later stages of tumour growth and development. Activating mutations of *AKT3* may also lead to activation of this pathway, though mutations of *AKT3* have not been reported in uveal melanoma up till now.

3.6.4 The metastasis-associated gene BAP1

Somatic mutations in the ubiquitin carboxyl-terminal hydrolase of BRCA1-associated protein 1 (BAP1) were found in 84% of gene-expression class II uveal melanomas (Harbour et al., 2010). The *BAP*1-gene is located on chromosome 3p21.1 and the encoded protein is part of the ubiquitin proteasome system that has been implicated in other cancer types as well, such as lung, breast and renal cell carcinoma (Harbour et al., 2010; Jensen et al., 1998; Patel, M. et al., 2011; Wood et al., 2007). *BAP*1 is reported to participate in multiprotein complexes involved in regulation of expression of several other genes that regulate cellular processes (Patel, M. et al., 2011). Somatic *BAP*1 mutation was only found in 1 out of 26 investigated class I tumours against 26 out of 31 class II tumours. These mutations are thus suggested to occur later in uveal melanoma progression than for instance *GNAQ* mutations (Harbour et al., 2010).

3.6.5 Other investigated genes

Several candidate genes were proposed in uveal melanoma recently, such as *DDEF*1, *NBS*1, *HDM*2, *LZST*-1, *APITD*1, *CCND*1 and *BCL*-2 (van den Bosch et al., 2010). For most of these genes, a definite role in tumourigenesis or progression towards metastasis has to be validated.

In 65% of uveal melanoma cases, *CCND*1 is reported to be overexpressed resulting in activation of cyclin dependent kinases (Coupland et al., 1998; Coupland et al., 2000; Ehlers & Harbour 2006). The *CCND*1 overexpression is associated with large tumour size, epitheloid cytology, and poor prognosis (Coupland et al., 2000).

Elevated expression of *BCL*-2 is observed in uveal melanomas but also in normal melanocytes. This overexpression is reported to block apoptosis (Brantley & Harbour 2000; Chana et al., 1999; Coupland et al., 2000; Jay et al., 1996) and suggested to be responsible for the resistance to chemotherapy or irradiation therapy (Ehlers & Harbour 2006; McGill et al., 2002).

3.7 Gene targeted therapy

The survival rates for patients with metastasised uveal melanoma, treated by chemotherapeutic drugs or combination chemotherapy regimens remain disappointingly low and toxicity may be significant (Sullivan & Atkins 2010). Conventional cytotoxic chemotherapeutics are toxic to all cells including normal cells, and therefore targeted therapy may be more valuable in the treatment of these patients. As the molecular basis for tumour development and progression is emerging, therapy aimed at interfering with specific molecular pathways may be important (Triozzi et al., 2008).

MAPK pathway activation appears to be important in uveal melanoma, therefore inhibition of this pathway or intermediates of this pathway represent a promising target (Sisley et al., 2011). $GNAQ^{Q209}$ mutations are exclusive to melanocytic tumour cells thereby enabling therapy specifically targeted at mutant cells only. *GNAQ* mutant cell lines appeared highly sensitive to inhibitors of MEK (Van Raamsdonk et al., 2009) and phase II clinical trials testing this hypothesis are currently underway (Sullivan & Atkins 2010).

Inhibitors of members of the RAS-RAF-MEK-ERK pathway such as the small-molecule inhibitor PLX4032, showed promising results in patients with cutaneous melanoma containing *BRAF* mutations. Tumour shrinkage was found in 80% of patients who received PLX4032 and progression-free survival was found to increase by an average of 7 months (Bollag et al., 2010; Flaherty et al., 2010; Herlyn & Nathanson 2010). These mutations are however rarely encountered in uveal melanoma patients. Downstream effectors of GNAQ and GNA11 remain to be elucidated and are highly potential targets for therapy. Care needs to be taken as interference with the normal function of these proteins, could be harmful. GNAQ protein activity for instance, appeared to be crucial for cardiomyocyte survival in animal models (Sisley et al., 2011). This problem may however be solved if inhibitors could be designed that specifically interfere with mutant *GNAQ* only. As MEK inhibitors proved to be of value for uveal melanoma, other ways to circumvent the *GNAQ*-protein blocking problem may lie in designing inhibitors of intermediates of the MAPK pathway such as BRAF, RAS, MEK and ERK.

IGF-1R and the downstream molecule mTOR, may also be involved in the PI3K-PTEN-AKT pathway and RAS-RAF-MEK-ERK pathway, and also serve as potential targets for inhibitor-therapy, which is currently being tested (Patel, M. et al., 2011).

New immunotherapeutic approaches are also currently tested, such as those administering patients immunomodulatory monoclonal antibodies (e.g. CTLA4 antibodies) or vaccinating

patients with their own dendritic cells trained to identify (circulating) tumour cells and initiate tumour cell desctruction by presenting tumour particles to cytotoxic T cells. We use this Dendritic Cell Therapy to treat high-risk patients after they have received local therapy, such as enucleation or irradiation, in order to eliminate circulating tumour cells and micrometastatic lesions. Results have to be awaited from this phase I study that already showed promising results in cutaneous melanoma patients (De Vries et al, 2005). Combination strategies such as immmunotherapy gene therapy may be more effective than single therapy regimes but these combinations have to be researched in the future.

4. Conclusion

Uveal melanoma is a rare but aggressive intraocular malignancy leading to metastatic spread in approximately 50% of patients. Current therapies have up till now unfortunately not resulted in improved survival. Patients with metastases from uveal melanoma still have a poor prognosis, with no effective adjuvant therapy available yet. Even chemotherapeutic agents administered alone or in combination, have not resulted in a change in survival rates for these patients. Recent cytogenetic and molecular genetic research identified several genetic prognostic factors, capable of making reliable predictions of prognosis in patients with uveal melanoma. These genetic factors prove to be even more important predictors than clinical and pathological factors and have already been implemented in the current ocular oncology clinical practice.

Next generation genetic techniques such as SNP-array, Next Generation Sequencing and gene-expression profiling shed light on chromosomal regions, genes, gene-expression, and molecular pathways involved in uveal melanoma tumour progression and development. More knowledge has been gained by these recent techniques combined with fine-needle aspiration biopsy tumour sampling, identifying the molecular genetic make-up of small and medium-sized melanomas as well as large melanomas. Uveal melanoma has hereby been identified as a heterogeneous type of malignancy showing variations in chromosome 3 alterations within tumours and variation in genes altered in different patients. With the molecular background of large and smaller-sized uveal melanoma emerging, patients may be selected on this molecular basis for future therapy.

Gene-targeted therapy is recently been tested in the clinical setting, facilitating interference with specific molecular pathways or signaling molecules, either as single agent or in combination with immunotherapy or chemotherapy. These developments may serve as first steps towards more specific and patient-tailored therapy not limited to treatment of patients with metastatic disease alone. These therapies may also be valuable for patients with recent diagnosis of uveal melanoma, attacking micrometastatic disease as early as possible.

There is great optimism that more specific and thus more effective therapies in the next several years will lead to advanced patient management, and thereby improved survival rates for patients with this deadly disease.

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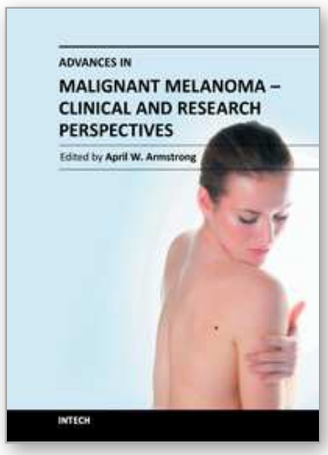

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This book titled Advances in Malignant Melanoma - Clinical and Research Perspectives represents an international effort to highlight advances in our understanding of malignant melanoma from both clinical and research perspectives. The authors for this book consist of an international group of recognized leaders in melanoma research and patient care, and they share their unique perspectives regarding melanoma epidemiology, risk factors, diagnostic and prognostic tools, phenotypes, treatment, and future research directions. The book is divide into four sections: (1) Epidemiology and Risk Factors of Melanoma, (2) Clinical Phenotypes of Melanoma, (3) Investigational Treatments for Melanoma and Pigmentary Disorders, and (4) Advances in Melanoma Translational Research. This book does not attempt to exhaustively cover all aspects of the aforementioned topics. Rather, it is a compilation of our authorsâ€™ pearls and unique perspectives on the relevant advances in melanoma during the recent years.

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