Genetic prognostication in uveal melanoma

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Chapter 6

Differential expression of DNA repair genes in prognostically-favorable versus prognostically-unfavorable Uveal Melanoma

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Manuscript submitted for publication

ABSTRACT

Purpose: To study the expression pattern of DNA repair genes in uveal melanoma (UM) and to identify genes that are differentially expressed between tumors with a favorable and an unfavorable prognosis.

Methods: Gene-expression profiling using the Illumina HT-12v4 chip was performed in 64 primary UM enucleated at the Leiden University Medical Center, The Netherlands, between 1999 and 2008. The expression pattern of 121 genes encoding proteins involved in DNA repair pathways was analyzed and the expression of 44 genes with a variable expression compared between disomy 3 and monosomy 3 tumors. Results were validated in a cohort from Genoa & Paris and the TCGA cohort. The effect of DNA-PKcs inhibition on cell survival was evaluated in UM cell lines.

Results: *PRKDC, WDR48, XPC,* and *BAP1* were the only genes significantly associated with clinical outcome after validation. Low expression of *WDR48* and *XPC* was related to large tumor diameter ($p=0.01$ and $p=0.004$, respectively), and a mixed/epithelioid cell type ($p=0.007$ and $p=0.03$, respectively). *PRKDC* was highly expressed in metastasizing UM ($p<0.001$), whereas *WDR48, XPC* and *BAP1* were lowly expressed ($p<0.001$, $p=0.006$, $p=0.003$, respectively). Pharmacological inhibition of DNA-PKcs resulted in decreased survival of UM cells.

Conclusions: *PRKDC* is significantly higher expressed in UM with an unfavorable prognosis, whereas the expression of *WDR48, XPC,* and *BAP1* is significantly lower in these tumors. *PRKDC* may be involved in proliferation, invasion and metastasis of UM cells. Unraveling the role of DNA repair genes may enhance our understanding of UM biology and result in the identification of new therapeutic targets.

INTRODUCTION

Uveal melanoma (UM) is an ocular malignancy that arises from melanocytes residing in the uveal tract, which consists of the iris, ciliary body and choroid. It is the second most common type of melanoma and the most common primary intraocular malignancy in adults, affecting approximately 5.1 individuals per million per year; it is most frequent in Caucasians,[1, 2] as a fair skin and light eye color have been identified as host susceptibility factors.[3, 4]

In general, local tumor control is excellent, with large primary ocular melanoma being treated by enucleation, and small to medium-sized tumors by application of a radioactive plaque, stereotactic irradiation, or proton beam therapy.[5-10] Despite excellent regional tumor control, UM is still often lethal: up to 50% of patients will develop metastatic disease, for which no effective treatment exists.[11] The liver is involved in approximately 90% of cases with metastasized disease.[12] Metastatic disease may develop at any time from the initial diagnosis of the primary tumor to several years after diagnosis.[13]

Several characteristics of the primary tumor are known to be associated with an infaust prognosis. These include a large size, ciliary body involvement, epithelioid cell type, extrascleral invasion and the presence of extravascular matrix loops.[14-18] Furthermore, specific genetic features, such as monosomy 3, amplification of chromosome 8q, and loss of chromosome 1p, correlate with a poor survival.[19-23] In contrast, an additional copy of chromosome 6p is associated with a favorable prognosis.[24, 25] Microarray gene expression analyses have resulted in the identification of two classes of UMs: class 1 tumors have low metastasic risk, while class 2 tumors are associated with a higher rate of metastatic death.[26-28] Recently, mutations in specific genes such as *BAP1* (BRCA1 associated protein-1), *SF3B1* (splicing factor 3b subunit 1), and *EIF1AX* (eukaryotic translation initiation factor 1A, X-linked) have been reported to have prognostic value.[29-31]

A lot of recent research in UM has focused on genetics, with the aim of unravelling UM biology and identifying specific aberrations that underlie the development of UM and may be potential targets of therapy.[29-32] An underexposed aspect of UM is the role of DNA repair in tumor development and progression. Aberrant DNA repair plays a role in the development of many malignancies and accordingly, genomic instability is considered a hallmark of cancer cells.[33] BAP1 protein, of which the loss of expression is related to a poor prognosis in UM,[34] has been shown to promote DNA double-strand break repair.[35] Although counterintuitive, DNA repair proteins in compensating pathways on

which tumor cells that have lost a repair pathway (over)rely (principle of synthetic lethality), may be targets for cancer therapeutics.[36, 37] Blocking DNA repair proteins to decrease the ability of UM cells to repair DNA damage may be used to sensitize tumors to traditional anti-cancer treatment by chemotherapy or radiotherapy.[38]

The lack of knowledge about the expression of DNA repair molecules in UM hampers such approaches in this malignancy. It is not yet known whether and how the DNA repair pathways are involved in the initiation and progression of UM. We, therefore, set out to analyze the expression of genes involved in DNA repair in UM and a putative association with different prognostic types of UM.

The main aim of this study was to determine the expression of genes involved in the different DNA repair pathways in UM. To test our hypothesis that genes involved in DNA repair are differentially expressed between tumors with a favorable and unfavorable prognosis, we determined the expression of these genes in 64 UMs and made a comparison between tumors with and without loss of chromosome 3. Additionally, the relation with survival was evaluated for differentially-expressed genes. Interesting associations were validated in two other sets of UM, and a potential druggable target was explored further.

METHODS

Study population

Our 'test set' contained 64 UMs obtained by primary enucleation at the Leiden University Medical Center (LUMC), Leiden, The Netherlands, between 1999 and 2008. Patient and tumor characteristics are shown in Table 1. Sufficient frozen material was available of these tumors and DNA of adequate quality could be retrieved. Survival data was retrieved from the patients' charts and from the Netherlands Comprehensive Cancer Organisation (https://iknl.nl/over-iknl/about-iknl), and updated in March 2017. In The Netherlands, general physicians report every cancer patient to the Netherlands Comprehensive Cancer Organisation, which collects and registers information on the survival status by contacting the general physicians yearly. The follow-up in The Netherlands is not intensive because of a lack of effective treatments for UM metastases and patients are often referred back to their general physician after treatment of the primary tumor. The median follow-up time was 62 months and no patient was lost to follow-up.

Table 1. Baseline characteristics of the test and validation sets. Percentages are rounded and may not equal 100.

CHARACTERISTIC	LUMC COHORT (n=64) Test set	GENOA & PARIS COHORT (n=110) Validation set	TCGA COHORT (n=80) 2nd Validation set
Gender			
Female	31 (48%)	41 (38%)	35 (44%)
Male	33 (52%)	67 (62%)	45 (56%)
Median age at enucleation/diagnosis (TCGA) (range)	61.6 (12.8 – 88.4) years	63.0 (29.0– 85.0) years	61.5 (22.0 – 86.0) years
Median LBD (range)	13.0 (8.0 – 30.0) mm	15.0 (2.0 – 23.0) mm	16.8 (10.0 – 23.6) mm
Median prominence (range)	8.0 (2.0 – 12.0) mm	11.1 (3.0 – 17.0) mm	11.0 (4.4 – 16.0) mm
AJCC size categories			
T1	6 (9%)	1 (1%)	0 (0%)
T2	25 (39%)	24 (27%)	14 (18%)
T3	31 (48%)	39 (44%)	32 (40%)
T4	2 (3%)	25 (28%)	34 (43%)
Cell type			
Spindle	22 (34%)	10 (12%)	43 (54%)
Mixed/Epithelioid	42 (66%)	71 (88%)	37 (46%)
Chromosome 3 status			
No monosomy 3	24 (38%)	46 (48%)	43 (54%)*
Monosomy 3	40 (63%)	49 (52%)	37 (46%)
Metastasis			
No	27 (42%)	54 (49%)	53 (66%)
Yes	37 (58%)	56 (51%)	27 (34%)

*Symbols: * 4 tumors were isodisomy 3 tumors*

Validation of the data was performed using two independent cohorts: datasets from Genoa & Paris, and data of The Cancer Genome Atlas (TCGA) project.[39] The Genoa & Paris cohort consisted of the UM microarray datasets GSE22138,[40] GSE27831,[41] and GSE51880[42] and were obtained from Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo/). The datasets were combined and normalized as described.The study followed the tenets of the Declaration of Helsinki (World Medical Association of Declaration 1964; ethical principles for medical research

involving human subjects) and was approved by the Medical Ethics Committee of the LUMC.

Histologic examination

After opening the bulbus following enucleation, a part of the tumor was retrieved and snap frozen at -80 °C. The remaining tumor tissue was formalin fixed (4% neutral-buffered) and embedded in paraffin. Conventional histologic evaluation by an ophthalmic pathologist for confirmation of diagnosis and determination of characteristics was done. Parameters such as largest basal diameter (LBD, in millimeters), thickness (in millimeters), mitotic count (per 2 mm^2 at 40x magnification, 8 high-power fields), tumor location, cell type (assessed according to the Armed Forces Institute of Pathology atlas)[43] were evaluated on 4 µm thick haematoxylin and eosin-stained sections. The 7th edition of the AJCC Cancer Staging Manual[44] was used to stage tumors according to the TNM classification system.

Genetic analyses

DNA and RNA were isolated from fresh-frozen tissue. DNA for single nucleotide polymorphism (SNP) analysis was extracted with the QIAmp DNA Mini kit and RNA for gene-expression profiling with the RNeasy Mini Kit (both from Qiagen, Venlo, The Netherlands). SNP array analysis to determine the chromosome copy number was performed with the Affymetrix 250K_NSP microarray chip (Affymetrix, Santa Clara, CA, USA) on all 64 UMs and with the Affymetrix Cytoscan HD chip (Affymetrix) on the cell lines. Chromosome Analysis Suite (ChAS, version 2.0225) from Affymetrix was used to determine chromosome copy numbers. Gene-expression profiling at the transcriptional level was carried out on RNA of 64 UMs using 35244 probes from the Illumina HT-12v4 chip (Illumina, San Diego, CA, USA). RNA for real-time PCR analysis in cell lines was isolated using the SV total RNA isolation kit (Promega, Madison, WI, USA), after which cDNA was synthesized using the reverse transcriptase reaction mixture as indicated by Promega. qPCR was performed using SYBR green mix (Roche Diagnostics, IN, USA) in a C1000 touch Thermal Cycler (Bio-Rad laboratories, Hercules, CA, USA). Relative expression of *PRKDC* and *SNAIL1* was determined compared to housekeeping genes *CAPNS1* and *SRPR*. Untreated samples average was set at 1.
RNAseq analysis in the cell lines was conducted at Institut Curie (Paris, France) after isolation of total RNA using a NucleoSpin Kit (Macherey-Nagel). cDNA

synthesis was conducted with MuLV Reverse Transcriptase in accordance with the manufacturers' instructions (Invitrogen, Carlsbad, CA, USA), with quality assessments conducted on an Agilent (Santa Clara, CA, USA) 2100 Bioanalyzer. Libraries were constructed using the TruSeq Stranded mRNA Sample Preparation Kit (Illumina) and sequenced on an Illumina HiSeq 2500 platform using a 100-bp paired-end sequencing strategy. TopHat (v2.0.6)[45] was used to align the reads against the human reference genome Hg19 RefSeq (RNA sequences, GRCh37) downloaded from the UCSC Genome Browser (http://genome.ucsc.edu). Gene expression was determined by featureCounts and normalized using DESeq2.

Gene Selection Procedure

We identified 121 genes encoding proteins involved in DNA repair mechanisms, based on a literature review on DNA repair, using the platforms Gene, OMIM, KEGG and PubMed. As our goal was to identify genes with a variable expression level, we determined the standard deviations of the expression levels of the DNA repair gene probes on the Illumina chip (n=178) (Supplementary Table S1). Certain genes were analyzed multiple times because they are encoded by different Illumina probes (in that case the distinction between probes is made by placing letters in alphabetic order at the end of the gene name), while 18 genes were not analyzed since they were not on the Illumina chip. A selection of genes was made based on a cut-off value of the standard deviation of the expression (Figure 1). A cut-off value of > 0.5 would result in 6 genes, of > 0.4 in 15 genes and a cut-off value of > 0.3 would lead to a total of 44 genes (encoded by 49 probes). A cut-off value of > 0.3 was chosen to have a reasonably-sized group of genes with still an acceptable level of variation in expression. The median expression of the probes of these 44 genes was compared between disomy 3 (D3) and monosomy 3 (M3) tumors and corrected for multiple testing using the Bonferroni method. 13 Genes which were significantly differentially expressed after Bonferroni correction were selected for further analysis.

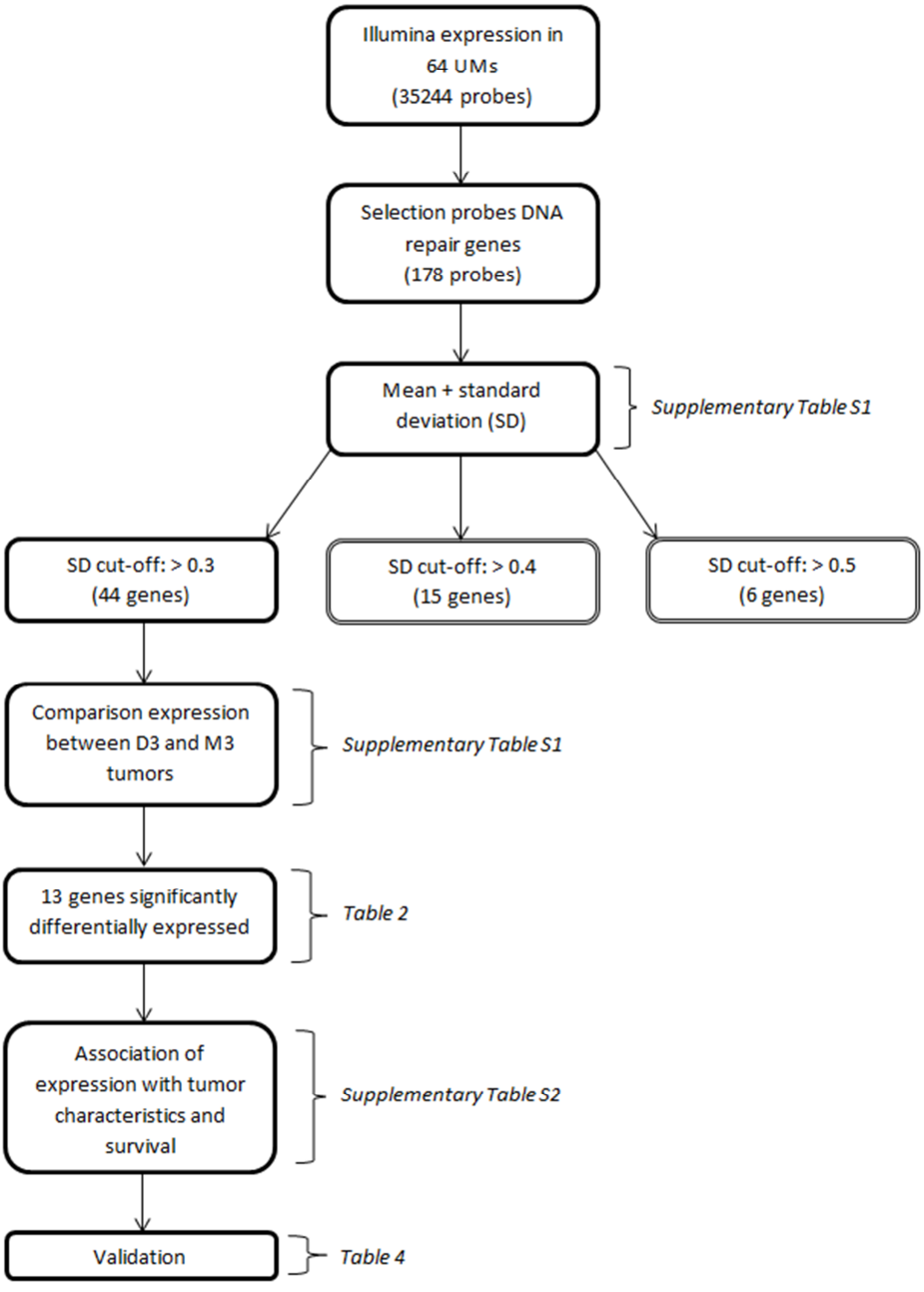


Figure 1. Flow-chart depicting all conducted analyses. Parentheses indicate the tables in which the results of the respective analyses are presented.

Cell lines, DNA-PKcs inhibition, and proliferation assay

Cell lines OMM2.5 (originally called OMM1.5) and Mel270, which are derived from the same patient, were obtained from Dr. Bruce Ksander[46] and maintained in RPMI supplemented with 10% FBS and antibiotics. MM28 was obtained from Dr. Sergio Roman-Roman[47] and grown in IMDM supplemented with 20% FBS (fetal bovine serum) and antibiotics. The OMM1 cell line was established by Dr. Gré Luyten.[48]

MM28 cells lack BAP1 expression, whereas Mel270, OMM2.5, and OMM1 cells are BAP1-positive.To evaluate the effect of DNA-PKcs inhibition on the expression of pro-metastatic factors, the expression of these factors was evaluated in a primary UM cell line (Mel270) and in a metastatic UM cell line (MM28) before and after treating the cells with 10μM NU7026 (#13308, Cayman Chemical, USA, stock concentration 20mM in DMSO) for 5 days. To analyze the effect of the DNA-PKcs inhibitor on growth of these UM cell lines, the cells were seeded in triplicate in 96-well plates. Treatment with NU7026 was started the next day. Cells were replenished with fresh medium with or without drugs after three days. Relative survival was determined after five days with the use of the CellTitreBlue assay (Promega) according to the manufacturer's protocol.

Statistical analysis

For data analysis, we used the statistical programming language R version 3.0.1 (R: A Language and Environment for Statistical Computing, R Core Team, R foundation for Statistical Computing, Vienna, Austria, 2014, http://www.R-project.org) supplemented with specialized packages for SNP and RNA analysis. The main package used for SNP analysis was aroma.affymetrix, supported by 'DNAcopy' (Venkatraman E. Seshan and Adam Olshen, DNAcopy: DNA copy number data analysis. R package version 1.34.0), 'sfit' (Henrik Bengtsson and Pratyaksha Wirapati (2013), sfit: Multidimensional simplex fitting. R package version 0.3.0/r185, http://R-Forge.R-project.org/projects/matrixstats/), and 'R.utils' (Henrik Bengtsson (2014), R.utils: Various programming utilities, R package version 1.29.8, http://CRAN.R-project.org/package=R.utils). The 'Aroma.Affymetrix' package made it possible to use the information from the SNP microarrays to determine copy number values. [49-51]

The packages used for RNA microarray analysis were 'limma' version 3.16.8, and the specific packages for Illumina microarrays: 'lumi' version 2.12.0, 'annotate' (R. Gentleman, annotate: Annotation for microarrays, R package version 1.38.0), and

the database package 'IlluminaHumanv4.db' (Mark Dunning, Andy Lynch and Matthew Eldridge, IlluminaHumanv4.db: Illumina HumanHT12v4 annotation data (chip IlluminaHumanv4), R package version 1.18.0).
The statistical software package SPSS v.20.0.0 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA) was used for data analysis. Population characteristics were described using medians and percentages. The Mann-Whitney U test was performed to analyze numerical variables between two groups, and the Kruskal-Wallis test in case more than two groups were compared. Kaplan-Meier survival curves were made and the log rank test was used to analyze significance. Differences were considered to be significant if $p<0.05$ after correction for multiple testing.

RESULTS

Population characteristics

Our cohort comprised 64 UM patients who had undergone primary enucleation at a median age of 61.6 years and of whom 33 (52%) were males (Table 1). The median LBD was 13 mm and the median thickness 8 mm. Most tumors were either classified as AJCC tumor size T2 (39%) or T3 (48%). A mixed/epithelioid cell type was recorded in 66% of cases. Monosomy 3 was detected in 63% of the tumors. At last follow-up, 37 (58%) patients had developed clinical metastases. We validated our data using two other independent cohorts: a set of 110 tumors from Genoa[41] and Paris[40], and the 80 UMs of The Cancer Genome Atlas (TCGA) project.[39] The characteristics of all cohorts are depicted in Table 1.

Gene expression in relation to chromosome 3 status

As loss of one copy of chromosome 3 is a very important prognostic marker in UM, we searched for DNA repair-related genes that showed a differential expression between tumors with and without loss of one chromosome 3.
The median expression of the 44 genes of interest was calculated and compared between disomy 3 and monosomy 3 tumors. A significantly different expression was found for 13 genes: the expression of three genes (*CENPX, DDB1, PRKDC*) was significantly higher in monosomy 3 tumors (Table 2A), whereas ten genes (*APEX1, BAP1, CETN2, GTF2H4, MLH1, RMI2, RPA1, SEM1, WDR48, XPC)* showed a significantly lower expression in tumors with monosomy 3 (Table 2B).

Table 2. Gene expression in relation to chromosome 3 status. Only genes of which the expression differed significantly between disomy 3 and monosomy 3 tumors are shown. The Mann-Whitney U test was conducted, Bonferroni correction was applied.

2A: Higher expression in monosomy 3 tumors:

GENE	CHARACTERISTICS OF GENE		EXPRESSION Median (range)		*P* VALUES	
	Pathway	Chromosome location	Disomy 3 (n=24)	Monosomy 3 (n=40)	*P* value	Corrected *P* value
CENPX	FA	17q25.3	9.3 (8.9-10.3)	9.7 (9.0-10.6)	<0.001	<0.001
DDB1	NER	11q12.2	12.1 (11.3-13.0)	12.4 (11.7-13.0)	0.001	0.04
PRKDC	DSBR	8q11.21	8.0 (7.3-8.6)	8.8 (7.8-10.2)	<0.001	<0.001

2B: Lower expression in monosomy 3 tumors:

GENE	CHARACTERISTICS OF GENE		EXPRESSION Median (range)		*P* VALUES	
	Pathway	Chromosome location	Disomy 3 (n=24)	Monosomy 3 (n=40)	*P* value	Corrected *P* value
APEX1	BER	14q11.2	11.0 (9.9-11.4)	10.5 (9.6-11.4)	<0.001	0.004
BAP1	DSBR	3p21.1	8.0 (6.6-8.5)	7.4 (6.4-8.1)	<0.001	<0.001
CETN2	NER	Xq28	10.2 (9.7-11.2)	9.9 (9.3-10.7)	<0.001	0.002
GTF2H4	NER	6p21.33	8.5 (6.9-9.4)	7.9 (7.2-9.3)	<0.001	<0.001
MLH1	MMR/FA	3p22.2	8.2 (7.5-8.8)	7.8 (7.1-8.3)	<0.001	<0.001
RMI2	DSBR	16p13.13	7.2 (6.7-7.7)	6.9 (6.5-7.7)	<0.001	0.02
RPA1	DSBR/MMR/NER	17p13.3	8.7 (7.7-9.2)	8.3 (7.4-8.9)	0.001	0.04
SEM1	DSBR	7q21.3	7.7 (7.3-8.4)	7.4 (6.8-8.0)	<0.001	0.01
WDR48	FA	3p22.2	8.2 (7.4-8.6)	7.6 (7.2-8.2)	<0.001	<0.001
XPC	NER	3p25.1	9.2 (8.3-9.7)	8.6 (8.0-9.3)	<0.001	<0.001

Chromosome dose effect and expression levels

As we wondered how expression levels were regulated, we considered the possibility that loss of chromosomal material or chromosome amplifications might influence expression levels, as noticed previously for other genes.[52, 53]

We compared the gene expression levels of the 44 genes of interest with the SNP copy number value of the chromosome region harboring the gene. We divided tumors into three groups: no aberration in the specified chromosome area, duplication in the specified region or a deletion of the region of interest. The analysis was only reliable for genes located on chromosomes 3, 6 or 8, since SNP analyses of other chromosomes showed no aberrant copy number in most tumors. Four genes that are located on chromosome 3p (*BAP1*, *MLH1, WDR48, XPC*) showed an association between a decreased expression and monosomy 3, while a trend towards decreased expression was noted for *MBD4* (chromosome 3q) (Table 3). The genes *FANCE* and *GTF2H4* (chromosome 6p) showed a significantly increased expression in tumors with gain of 6p, while for *GTF2H5* (chromosome 6q) a significantly lower expression was found in tumors with loss of 6q. The expression of *POLB* (chromosome 8p) was significantly decreased in tumors with loss of 8p, while an increased expression of *NBN* and *PRKDC*, which are located on the long arm of chromosome 8, was related to gain of genetic material in that chromosome region.

Gene expression in relation to histological data and survival

The expression of the 13 genes, that were found to be differentially expressed between disomy 3 and monosomy 3 tumors, was compared to histopathological data and survival (Supplementary Table S2).

With regard to associations between gene expression and tumor diameter, we noticed an association between low expression of *WDR48* and *XPC* and a large LBD (p=0.01 and p=0.004, respectively), while a high expression of *CENPX* correlated with a large LBD (p=0.02). Although the difference in expression was small, *CENPX* showed a significantly higher expression in tumors with a mixed/epithelioid cell type (p=0.04). In contrast, the expression of the genes *WDR48* (p=0.007) and *XPC* (p=0.03) was significantly lower in cases with a mixed/epithelioid cell type.

Regarding AJCC staging, the expression of *CENPX* (p=0.01) was significantly higher in tumors with higher AJCC stages, while the expression of the *RPA1* gene (p=0.03) was significantly lower in cases with a higher AJCC stage.

Table 3. The relation between chromosome dose and gene expression for genes of the group of 44 genes of interest. The analysis was only reliable for genes located on chromosomes 3, 6 or 8, since other chromosomes don't show frequent aberrations in UM.

GENE	CHARACTERISTICS OF GENE		EXPRESSION Median (range)		*P* VALUE
	Pathway	Chromosome location	No aberrant copy number	Aberrant copy number *chromosome 3: loss* *chromosome 6p: gain* *chromosome 6q: loss* *chromosome 8p: loss* *chromosome 8q: gain*	
BAP1	DSBR	3p21.1	8.0 (6.6 – 8.5) *n=24*	7.4 (6.4 – 8.1) *n=40*	<0.001
FANCE	FA/DSBR	6p21.31	7.4 (6.8 – 8.2) *n=43*	7.9 (6.9 – 8.6) *n=21*	<0.001
GTF2H4	NER	6p21.33	7.9 (6.9 – 8.5) *n=43*	8.5 (7.8 – 9.4) *n=21*	<0.001
GTF2H5	NER	6q25.3	10.3 (9.4 – 11.2) *n=53*	9.9 (9.3 – 10.4) *n=11*	0.004
MBD4	BER/DSBR	3q21.3	8.4 (7.5 – 9.9) *n=24*	8.1 (7.5 – 9.3) *n=40*	0.33
MLH1	MMR/FA	3p22.2	8.2 (7.5 – 8.8) *n=24*	7.8 (7.1 – 8.3) *n=40*	<0.001
NBN	DSBR	8q21.3	7.9 (7.3 – 8.3) *n=19*	8.2 (7.4 – 9.2) *n=45*	<0.001
POLB	BER	8p11.21	10.0 (8.6 – 10.9) *n=49*	8.9 (8.1 – 10.2) *n=15*	<0.001
PRKDC	DSBR	8q11.21	8.0 (7.5 – 8.5) *n=19*	8.7 (7.3 – 10.2) *n=45*	<0.001
WDR48	FA	3p22.2	8.2 (7.4 – 8.6) *n=24*	7.6 (7.2 – 8.2) *n=40*	<0.001
XPC	NER	3p25.1	9.2 (8.3 – 9.7) *n=24*	8.6 (8.0 – 9.3) *n=40*	<0.001

The genes *CENPX* and *PRKDC* were highly expressed in tumors that gave rise to metastases (both p<0.001). In contrast, the genes *BAP1, CETN2, GTF2H4, MLH1*, *RMI2, SEM1, WDR48, and XPC* showed a lower expression in the metastasis group (Mann-Whitney U test).

Considering survival, two genes were associated with poor survival when highly expressed: *CENPX* and *PRKDC*. In contrast, a low expression of the genes *BAP1, GTF2H4, RMI2, SEM1, WDR48,* and *XPC* was associated with an unfavorable prognosis (log-rank test).

Validation

Validation was performed by analyzing the expression levels of the 13 genes of interest in two other sets of tumors: a set of 110 tumors from Genoa and Paris and another set of 80 UMs of The Cancer Genome Atlas (TCGA) project.[39] In each validation set, median expression levels for every gene were calculated to establish two groups of tumors for Kaplan-Meier analyses. The occurrence of metastases was the event of interest in the tumors from Genoa and Paris (taken together), while death due to UM metastases was the endpoint of analysis for the TCGA tumors. In the Genoa and Paris set, more than one p-value is presented for some genes, since these genes were analyzed several times using different probes. The association of the expression of a gene with survival was considered 'validated' provided that in all three sets (LUMC set and the two validation sets) a significant association was observed. Of the 13 genes, four were significantly associated with survival in all three cohorts. A high expression of *PRKDC* was associated with poor survival, as was a low expression of *BAP1, WDR48,* and *XPC* (Table 4). Survival curves for these genes in patients from the LUMC cohort are shown in Figure 2. As cut-off value, we used the median expression of each gene.

PRKDC

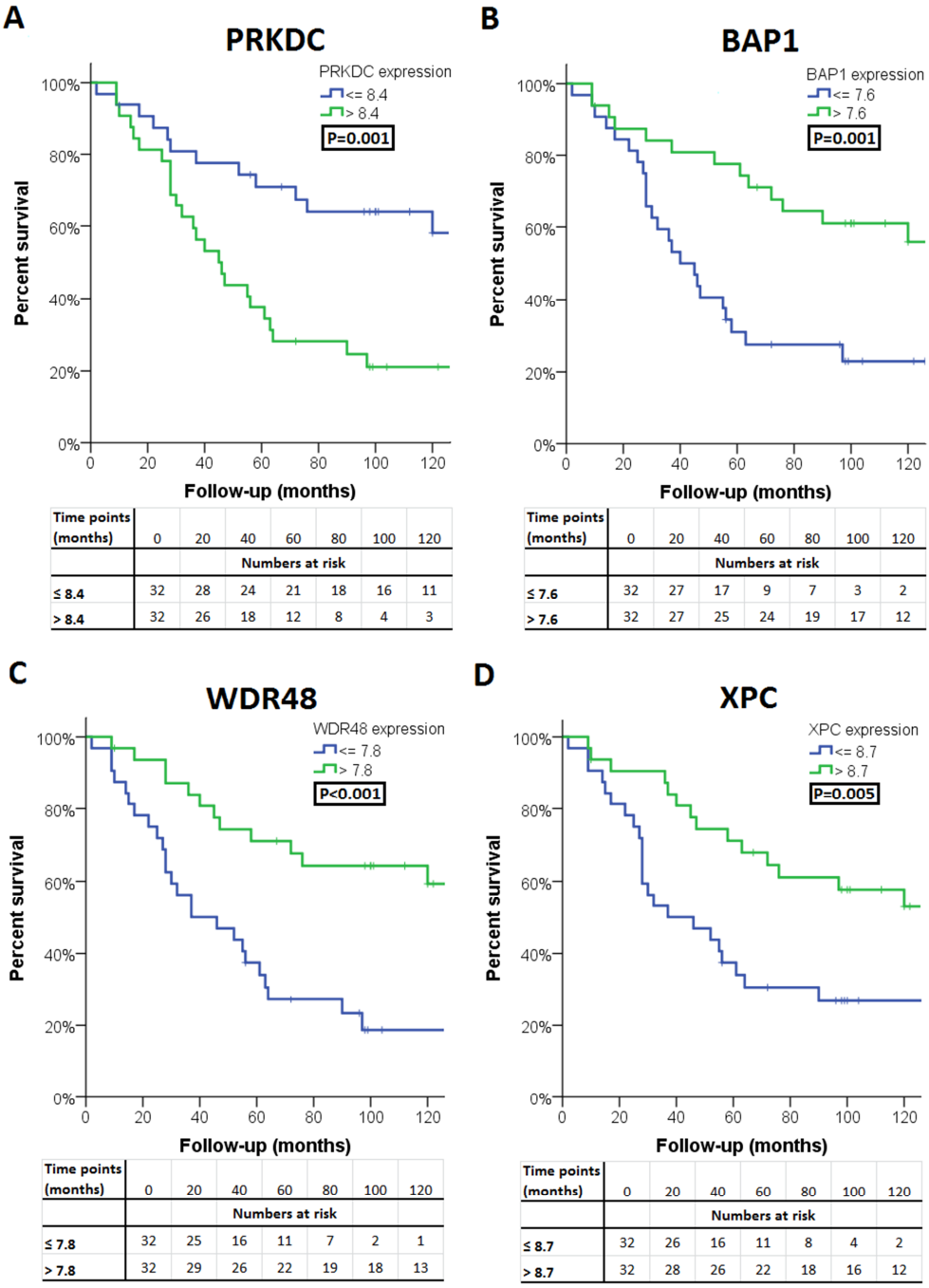
Because of our finding that a high expression of the *PRKDC* gene located on chromosome 8q is related to an unfavorable prognosis and the fact that gain of material of chromosome 8q predicts an adverse clinical outcome, we decided to perform further (experimental) analyses to study the biological significance of the *PRKDC* gene in UM. Our decision to focus on *PKRDC* was furthermore fuelled by the finding that the DNA-PKcs protein encoded by *PRKDC* has been shown to modulate cell survival, proliferation, invasion and migration in other cancers.[54, 55] First, we analyzed the relation between chromosome 8q copy number variation and *PRKDC* expression in the LUMC and the TCGA cohort. This analysis could not be performed for the Genoa and Paris cohort because the chromosome 8q status of these tumors was unknown. A higher chromosome 8q copy number was significantly correlated to a higher expression of *PRKDC* in the LUMC cohort (correlation coefficient: 0.67, $p<0.001$) as well as the TCGA cohort (correlation coefficient: 0.61, $p<0.001$) (Figure 3). We also analyzed the association between 8q copy number and *PRKDC* expression, determined by RNAseq in 12 UM cell lines, and by qPCR in 13 UM cell lines (Figure 4).

Table 4. Validation of the 13 genes which were significantly differentially expressed between disomy 3 and monosomy 3 tumors in the LUMC cohort. Validation was performed in an independent cohort of 110 tumors (Genoa + Paris) and in the TCGA cohort of 80 tumors. Shown are p-values of the log-rank test. Significant p-values are in bold. Genes that are significantly associated with survival in all cohorts are depicted in the last column.

GENE	CHARACTERISTICS OF GENE		LUMC COHORT (n=64) Test set	GENOA & PARIS COHORT (n=110) Validation set	TCGA COHORT (n=80) 2nd Validation set	VALIDATED GENES
	Pathway	Chromosome location				
CENPX	FA	17q25.3	**<0.001**	0.09	**0.03**	
DDB1	NER	11q12.2	0.48	0.75	0.22	
PRKDC	DSBR	8q11.21	**0.001**	**0.005/0.01/<0.001**	**0.002**	***PRKDC****
APEX1	BER	14q11.2	0.05	0.77	**0.04**	
BAP1	DSBR	3p21.1	**0.001**	**<0.001**/0.15	**<0.001**	***BAP1***†
CETN2	NER	Xq28	0.18	**0.04**	**0.001**	
GTF2H4	NER	6p21.33	**0.001**	0.97	**0.001**	
MLH1	MMR/FA	3p22.2	0.07	**0.005**	0.08	
RMI2	DSBR	16p13.13	**0.02**	0.63	**0.005**	
RPA1	DSBR/ MMR/NER	17p13.3	0.41	0.39/0.26/**0.002**	**0.04**	
SEM1	DSBR	7q21.3	**0.006**	0.06	**0.02**	
WDR48	FA	3p22.2	**<0.001**	0.07/0.06/**0.04/0.03**	**0.003**	***WDR48***†
XPC	NER	3p25.1	**0.005**	**0.02**	**0.01**	***XPC***†

*Symbol: * higher expression associated with poor survival, † lower expression associated with poor survival*

Although the association was not significant (RNAseq: p=0.23, qPCR: p=0.2 [Kruskal-Wallis test]), we observed a trend towards higher expression of *PRKDC* in cell lines with more copies of 8q, which was in agreement with our findings in primary tumors (Figure 3). However, this association was less evident than in primary tumors, due to the lower number of cases and the lack of cell lines with 2 copies of chromosome 8q or more than 4 copies of 8q. The correlation was most pronounced in the RNAseq analysis (Figure 4A) and less clear in the qPCR analysis (Figure 4B), of which the correlation was slightly distorted by cell lines 92.1 and OMM2.5, which have 3 copies of chromosome 8q but show a *PRKDC* expression that is comparable to cell lines with 4 copies. However, there was a subpopulation of cells having 4 copies of chromosome 8q (indicating mosaicism) in cell line 92.1. To test our hypothesis that *PRKDC* is a possible driver of metastasis in UM, we wondered in which ways *PRKDC* could be involved in invasion and migration of UM cells.



Time points (months)	0	20	40	60	80	100	120
	Numbers at risk						
≤ 8.4	32	28	24	21	18	16	11
> 8.4	32	26	18	12	8	4	3

Time points (months)	0	20	40	60	80	100	120
	Numbers at risk						
≤ 7.6	32	27	17	9	7	3	2
> 7.6	32	27	25	24	19	17	12

Time points (months)	0	20	40	60	80	100	120
	Numbers at risk						
≤ 7.8	32	25	16	11	7	2	1
> 7.8	32	29	26	22	19	18	13

Time points (months)	0	20	40	60	80	100	120
	Numbers at risk						
≤ 8.7	32	26	16	11	8	4	2
> 8.7	32	28	26	22	18	16	12

Figure 2. Kaplan-Meier survival curves of the four genes of which the expression was significantly associated with clinical outcome in all three cohorts.

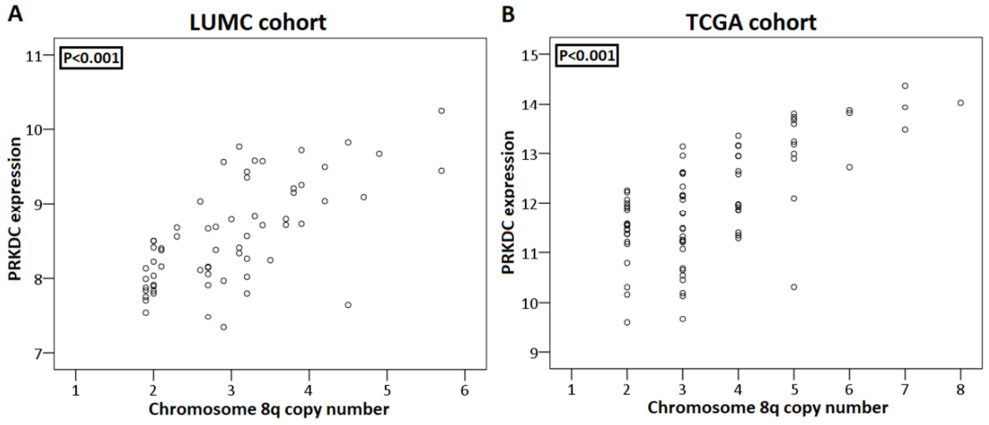


Figure 3. Correlation between PRKDC expression and chromosome 8q copy number in primary UM.

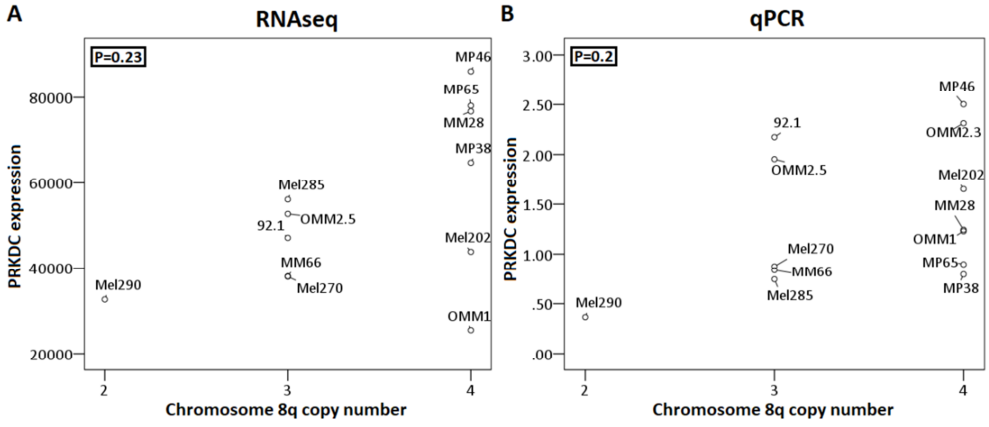


Figure 4. Correlation between PRKDC expression and chromosome 8q copy number in UM cell lines.

A study in prostate cancer showed that transcriptional regulation by the DNA-PKcs protein encoded by the *PRKDC* gene promotes invasion, migration and metastasis.[54] As the expression of *ZEB1*, *TWIST1* and *SNAIL1* have been proposed to play a role in invasion of UM cells,[56] we evaluated whether inhibition of DNA-PKcs influenced expression of these genes. The expression was evaluated in a primary UM cell line (Mel270) and in a metastatic UM cell line (MM28) before and after treating the cells with 10µM NU7026 for 5 days. The basal expression level of these genes was however low in both cell lines. Inhibition of DNA-PKcs by NU7026 led to a downregulation of *SNAIL1* in Mel270 as well as MM28 cells (Figure 5). *ZEB1* and *TWIST1* expression were not affected (data not shown).

To analyze the effect of DNA-PKcs inhibition on cell proliferation, we treated four cell lines (OMM1, OMM2.5, Mel270, MM28) with increasing doses of NU7026 up to 10 µM for a period of 5 days (Figure 6). All cell lines were to some extent affected by the DNA-PKcs inhibitor. The strongest growth inhibitory effect was noted in cell lines Mel270 and MM28 showing a 55% and 43% inhibition, respectively.

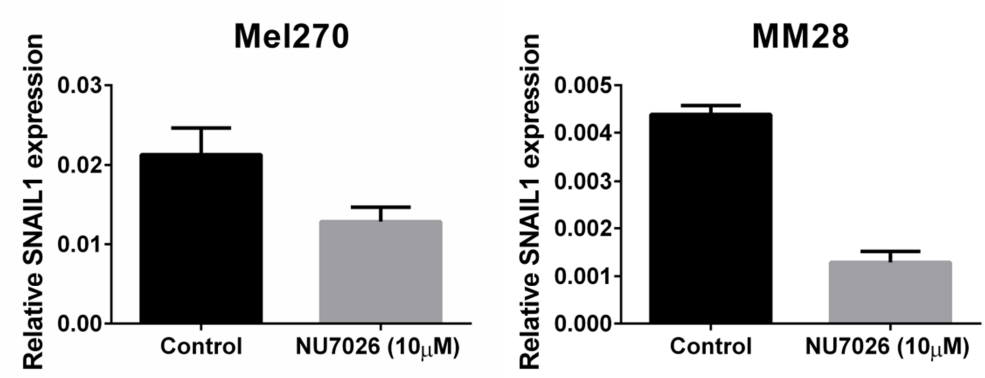


Figure 5. The effect of DNA-PKcs inhibition on the mRNA expression of *SNAIL1* in cell lines Mel270 and MM28. Cells were treated with 10 µM of the DNA-PKcs inhibitor NU7026 for 5 days.

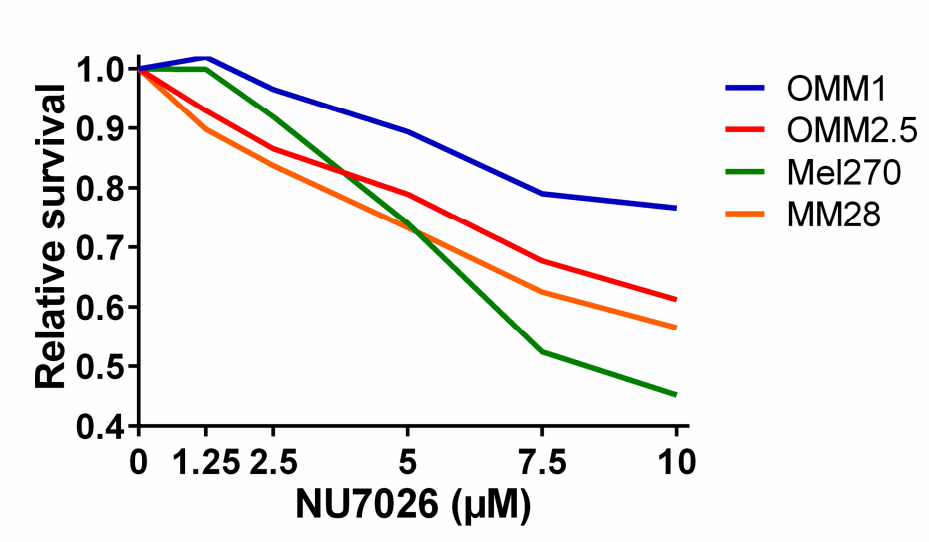


Figure 6. The relative survival in UM cell lines OMM1, OMM2.5, Mel270, and MM28 upon treatment with increasing doses of the DNA-PKcs inhibitor NU7026.

DISCUSSION

Biological cellular responses following DNA damage include DNA damage repair, damage tolerance, cell-cycle checkpoint control and apoptosis. These mechanisms are tightly regulated and which pathway becomes activated depends on the type and severity of the DNA damage. In case of severe damage, the complex signalling pathways may eventually arrest the cell cycle (providing more time for repair and tolerance mechanisms to act) or lead to apoptosis.[57, 58] The recognition of expression patterns of the genes involved in DNA repair in UM is the first step to understand the way these genes play a role in UM development and may help in identifying new targets for therapy.

In this study, we evaluated the expression of DNA repair-related genes in the Leiden cohort of 64 UMs and aimed to identify genes with a variable expression between prognostically-favorable and prognostically-unfavorable UM. After validation in two other independent cohorts, we identified 4 genes, which were associated with the degree of malignancy in UM: three genes (*BAP1, WDR48*, and *XPC1*) showed an association between a low expression and poor survival, while *PRKDC* was highly expressed in cases with an unfavorable prognosis. The genes *BAP1, WDR48*, and *XPC1* are all located on chromosome 3p and showed a significantly lower expression in monosomy 3 tumors. A lower expression of the *MLH1* gene, which is also located on chromosome 3p, was significantly related to prognosis in one cohort and showed a near-significant effect in the other cohorts. Since these four genes play a role in DNA repair, we can expect that impaired DNA repair is one of the results of the loss of a copy of chromosome 3. Sustained DNA damage as a result of deficient DNA repair mechanisms may lead to the accumulation of chromosomal abnormalities and gene mutations, which may promote cell growth and proliferation. Chromosome 3 loss does not occur in single step since small tumors with partial monosomy have been observed,[59] but apparently, loss of the entire chromosome confers a selective advantage that might be mediated by the DNA-repair genes identified here.

BAP1 (BRCA1-associated protein 1) is a gene located on chromosome 3p21.1. The *BAP1* gene encodes a nuclear ubiquitin carboxy-terminal hydrolase, which is a deubiquitinating enzyme.[60] It has been described to be a tumor suppressor gene that functions in the BRCA-1 control pathway. The BAP1 protein contains binding domains for BRCA1 and BARD1, enzymes that form a heterodimeric complex that functions as a tumor suppressor.[61] Loss of BAP1 has been shown to be related to a

poor clinical outcome in UM.[29, 39] Similarly, a lower gene expression of *BAP1* in our study corresponded to a poor survival.

Ubiquination and deubiquination regulate essential biological processes such as DNA replication and DNA repair.[61] In accordance, BAP1 has been shown to play a role in the repair of DNA double strand-breaks by homologous recombination.[35, 62]. It has been suggested that the DNA repair function of BAP1 may thus be the molecular basis for its tumor suppressor function in UM.[35]

Another DNA repair-related gene involved in deubiquitination which in our study showed a low expression in metastasizing uveal melanoma is *WDR48.* It is also known as *UAF1* and is located in close proximity (on 3p22.2) to *BAP1*. UAF1 forms a complex with USP1, a deubiquitinating enzyme, to form the UAF1/USP1 complex which regulates the Fanconi Anemia DNA repair pathway.[63] UAF1 exerts its function by activating USP1, and USP1 regulates the Fanconi Anemia repair pathway by deubiquitinating FANCD2, one of the most important players of this pathway. Fanconi Anemia is an inherited genomic instability disorder that led to the discovery of a novel DNA repair pathway. The Fanconi Anemia repair pathway plays a role in the repair of DNA cross-links and can be activated after various types of DNA damage, such as ionizing radiation and ultraviolet light.[64, 65] Accurate deubiquitination of the FANCD2 protein by the USP1/UAF1 complex is essential for an intact Fanconi Anemia pathway and a proper DNA damage repair.[66, 67] Because of this crucial role of the *WDR48* gene, and the association we found of a low expression of *WDR48* with poor prognosis, a defective Fanconi Anemia repair pathway may play a role in the malignant transformation of UM. Murine fibroblasts deficient in UAF1 have been shown to exhibit profound chromosomal instability.[68]

XPC (Xeroderma Pigmentosum, complementation group C) is the third gene located on chromosome 3p of which a low expression was associated with poor survival in our study. The *XPC* gene, located in the region 3p25.1, encodes a protein that helps to form the XPC repair complex and is involved in the early steps of the DNA Nucleotide Excision Repair (NER) pathway. Mutations in *XPC* that impair the production of XPC protein are related to Xeroderma Pigmentosum (XP), a rare recessive disorder, which makes patients extremely sensitive to ultraviolet light. This results in the frequent development of skin tumors mainly in areas of the body exposed to the sun. The XPC protein functions as a damage sensor detecting DNA damage.[69-72] The association of the low expression of *XPC* with poor survival in UM is interesting, since evidence for the association of ultraviolet

light exposure and UM development is inconclusive. However, XPC may play a role that is independent of its direct function related to UV-damage, as evidenced by the association of epigenetic silencing of *XPC* with shorter survival in bladder cancer.[73] Interestingly, the XPC repair complex contains the CETN2 protein, which shows a significantly lower expression in metastasizing UMs in the two validation cohorts of our study (Table 4).[74] Xeroderma Pigmentosum is associated with a higher risk for ocular malignancies.[75]

In contrast to the above discussed genes, the *PRKDC* gene which is located on chromosome 8q11.21 was found to be associated with worse survival when expressed highly.[76] *PRKDC* encodes the catalytic subunit of DNA-dependent serine/threonine protein kinase (DNA-PKcs). DNA-PK is involved in the repair of double strand breaks (DSBs) by non-homologous end-joining (NHEJ).[77-79] DSBs can develop due to the effects of reactive oxygen intermediates or by exogenous agents such as ionizing radiation and anticancer chemotherapeutic drugs.[80]

High expression of DNA repair proteins such as DNA-PKcs may increase the ability of tumor cells to withstand damage caused by chemotherapy or irradiation. Accordingly, increased DNA-PKcs activity was related to glioma resistance to cisplatin chemotherapy.[81] Moreover, upregulation of DNA-PKcs was detected after irradiation in oral squamous cell carcinoma (OSCC) that were resistant to radiotherapy. Targeting DNA-PKcs has been suggested as a novel sensitization therapy of OSCC and has been shown to increase anticancer drug sensitivity in osteosarcoma cell lines.[82, 83] Since the majority of primary UMs is treated by radiotherapy and certain chemotherapeutic targets are being tested for their effectiveness in killing UM metastases, elucidating the role of DNA-PKcs in UM may pave the way for sensitization therapy in UM by inhibiting DNA-PKcs.

We demonstrate that gain of chromosome 8q is related to a higher expression of *PRKDC* in our cases as well as in the TCGA cohort and UM cell lines. It is known that amplification of chromosome 8q is associated with an adverse clinical outcome in UM.[25, 84] Although the exact mechanisms by which gain of chromosome 8q confers its malignant effect has not yet been elucidated, overexpression of *DDEF1* has been suggested as a potential mechanism.[85] Interestingly, a recent study in prostate cancer has shown that the DNA-PKcs protein encoded by *PRKDC* modulates cell invasion and migration and functions as a potent driver of tumor progression and metastasis.[54] Activated DNA-PKcs has been correlated to increased proliferation, decreased apoptosis and poor survival in hepatocellular carcinoma.[55] In accordance, DNA-PKcs has been shown to be

involved in normal cell cycle progression by controlling proper chromosome segregation and cytokinesis.[86] In this study, we show that inhibition of DNA-PKcs is related to decreased proliferation of UM cells. The relative growth in four different UM cell lines decreased with increasing doses of NU7026, which is an inhibitor of DNA-PKcs.

A recent study by Kotula *et al.* in the cutaneous melanoma cell line SK28 demonstrated that DNA-PKcs has pro-metastatic activity by modulating the tumor microenvironment through controlling the secretion of e.g. matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs).[87] We found a low and variable expression of MMPs and TIMPs in the majority of UM cell lines we analyzed and did not observe an evident regulatory effect following DNA-PKcs inhibition (data not shown). Since DNA-PKcs is postulated to be a driver of invasion and metastasis, we analyzed the effect of DNA-PKcs inhibition on the expression of epithelial-to-mesenchymal transformation (EMT) – associated factors that have been shown to play a role in invasiveness of UM cells (*ZEB1, TWIST1, SNAIL1*).[56] Although the expression of these factors was low in the UM cell lines, we observed a decrease in the expression of the pro-metastatic *SNAIL1* upon DNA-PKcs inhibition. The inhibition of the protein interaction between DNA-PKCs and Snail1 has been suggested to be an effective strategy for inhibiting tumor migration.[88]

Considering the suggested pro-metastatic functions of DNA-PKcs, it is conceivable that an increased expression of *PRKDC* as a result of amplification of 8q may contribute to the malignant progression in UM. This would imply that DNA-PKcs could be a potential target for therapy in UM. Furthermore, the use of inhibitors of DNA repair proteins is a promising option for treating metastases, since cancer cells only retain some DNA repair modules and are dependent on these for survival.[89]

In conclusion, we show that several important DNA repair molecules are differentially expressed between tumors with good and adverse prognosis. Furthermore, we report on the effects of DNA-PKcs inhibition on cell survival and expression of pro-metastatic genes in UM cell lines. We suggest that DNA-PKcs, encoded by the *PRKDC* gene on chromosome 8q, may be involved in proliferation, invasion, and metastasis of UM cells and should be investigated further. An increased insight of factors involved in DNA repair mechanisms in uveal melanoma will hopefully enhance our understanding of the pathogenesis of this disease and may eventually result in the identification of new targets of therapy.

Conflicts of interest

All authors have declared that no conflicts of interest exist.

Grant support

This study was funded by research grants from 'Funding Program Science Without Borders, CNPq/CAPES, Brazilian Government', the Horizon 2020 UM CURE grant (European Union), The Eye Cancer Network (New York, NY, United States of America), and by the IG17103 grant from the Associazione Italiana per la Ricerca sul Cancro to UP.

REFERENCES

1. Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on Cancer and the American Cancer Society. *Cancer* 1998;83:1664-1678.
2. Singh AD, Turell ME, Topham AK. Uveal melanoma: trends in incidence, treatment, and survival. *Ophthalmology* 2011;118:1881-1885.
3. Holly EA, Aston DA, Char DH, Kristiansen JJ, Ahn DK. Uveal melanoma in relation to ultraviolet light exposure and host factors. *Cancer Res* 1990;50:5773-5777.
4. Weis E, Shah CP, Lajous M, Shields JA, Shields CL. The association between host susceptibility factors and uveal melanoma: a meta-analysis. *Arch Ophthalmol* 2006;124:54-60.
5. Diener-West M, Hawkins BS, Markowitz JA, Schachat AP. A review of mortality from choroidal melanoma. II. A meta-analysis of 5-year mortality rates following enucleation, 1966 through 1988. *Arch Ophthalmol* 1992;110:245-250.
6. Jampol LM, Moy CS, Murray TG, et al. The COMS randomized trial of iodine 125 brachytherapy for choroidal melanoma: IV. Local treatment failure and enucleation in the first 5 years after brachytherapy. COMS report no. 19. *Ophthalmology* 2002;109:2197-2206.
7. Krohn J, Monge OR, Skorpen TN, Mork SJ, Dahl O. Posterior uveal melanoma treated with I-125 brachytherapy or primary enucleation. *Eye (Lond)* 2008;22:1398-1403.
8. Tarmann L, Wackernagel W, Avian A, et al. Ruthenium-106 plaque brachytherapy for uveal melanoma. *Br J Ophthalmol* 2015;99:1644-1649.
9. Riechardt AI, Cordini D, Willerding GD, et al. Proton beam therapy of parapapillary choroidal melanoma. *Am J Ophthalmol* 2014;157:1258-1265.
10. Seddon JM, Gragoudas ES, Egan KM, et al. Relative survival rates after alternative therapies for uveal melanoma. *Ophthalmology* 1990;97:769-777.
11. Augsburger JJ, Correa ZM, Shaikh AH. Effectiveness of treatments for metastatic uveal melanoma. *Am J Ophthalmol* 2009;148:119-127.
12. Singh AD, Bergman L, Seregard S. Uveal melanoma: epidemiologic aspects. *Ophthalmol Clin North Am* 2005;18:75-84, viii.
13. Kujala E, Makitie T, Kivela T. Very long-term prognosis of patients with malignant uveal melanoma. *Invest Ophthalmol Vis Sci* 2003;44:4651-4659.
14. Kujala E, Damato B, Coupland SE, et al. Staging of ciliary body and choroidal melanomas based on anatomic extent. *J Clin Oncol* 2013;31:2825-2831.
15. Seddon JM, Albert DM, Lavin PT, Robinson N. A prognostic factor study of disease-free interval and survival following enucleation for uveal melanoma. *Arch Ophthalmol* 1983;101:1894-1899.
16. Affeldt JC, Minckler DS, Azen SP, Yeh L. Prognosis in uveal melanoma with extrascleral extension. *Arch Ophthalmol* 1980;98:1975-1979.

17. Makitie T, Summanen P, Tarkkanen A, Kivela T. Microvascular loops and networks as prognostic indicators in choroidal and ciliary body melanomas. *J Natl Cancer Inst* 1999;91:359-367.
18. Shields CL, Furuta M, Thangappan A, et al. Metastasis of uveal melanoma millimeter-by-millimeter in 8033 consecutive eyes. *Arch Ophthalmol* 2009;127:989-998.
19. Horsman DE, Sroka H, Rootman J, White VA. Monosomy 3 and isochromosome 8q in a uveal melanoma. *Cancer Genet Cytogenet* 1990;45:249-253.
20. Prescher G, Bornfeld N, Hirche H, Horsthemke B, Jockel KH, Becher R. Prognostic implications of monosomy 3 in uveal melanoma. *Lancet* 1996;347:1222-1225.
21. Sisley K, Rennie IG, Parsons MA, et al. Abnormalities of chromosomes 3 and 8 in posterior uveal melanoma correlate with prognosis. *Genes Chromosomes Cancer* 1997;19:22-28.
22. Kilic E, Naus NC, van Gils W, et al. Concurrent loss of chromosome arm 1p and chromosome 3 predicts a decreased disease-free survival in uveal melanoma patients. *Invest Ophthalmol Vis Sci* 2005;46:2253-2257.
23. Dogrusoz M, Jager MJ. Genetic prognostication in uveal melanoma. *Acta Ophthalmol* 2017.
24. White VA, Chambers JD, Courtright PD, Chang WY, Horsman DE. Correlation of cytogenetic abnormalities with the outcome of patients with uveal melanoma. *Cancer* 1998;83:354-359.
25. Damato B, Dopierala J, Klaasen A, van Dijk M, Sibbring J, Coupland SE. Multiplex ligation-dependent probe amplification of uveal melanoma: correlation with metastatic death. *Invest Ophthalmol Vis Sci* 2009;50:3048-3055.
26. Onken MD, Worley LA, Ehlers JP, Harbour JW. Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death. *Cancer Res* 2004;64:7205-7209.
27. Onken MD, Worley LA, Tuscan MD, Harbour JW. An accurate, clinically feasible multi-gene expression assay for predicting metastasis in uveal melanoma. *J Mol Diagn* 2010;12:461-468.
28. Onken MD, Worley LA, Char DH, et al. Collaborative Ocular Oncology Group report number 1: prospective validation of a multi-gene prognostic assay in uveal melanoma. *Ophthalmology* 2012;119:1596-1603.
29. Harbour JW, Onken MD, Roberson ED, et al. Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science* 2010;330:1410-1413.
30. Yavuzyigitoglu S, Koopmans AE, Verdijk RM, et al. Uveal Melanomas with SF3B1 Mutations: A Distinct Subclass Associated with Late-Onset Metastases. *Ophthalmology* 2016;123:1118-1128.

31. Ewens KG, Kanetsky PA, Richards-Yutz J, et al. Chromosome 3 status combined with BAP1 and EIF1AX mutation profiles are associated with metastasis in uveal melanoma. *Invest Ophthalmol Vis Sci* 2014;55:5160-5167.
32. Amaro A, Gangemi R, Piaggio F, et al. The biology of uveal melanoma. *Cancer Metastasis Rev* 2017;36:109-140.
33. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646-674.
34. Shah AA, Bourne TD, Murali R. BAP1 protein loss by immunohistochemistry: a potentially useful tool for prognostic prediction in patients with uveal melanoma. *Pathology* 2013;45:651-656.
35. Yu H, Pak H, Hammond-Martel I, et al. Tumor suppressor and deubiquitinase BAP1 promotes DNA double-strand break repair. *Proc Natl Acad Sci U S A* 2014;111:285-290.
36. Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA. DNA repair pathways as targets for cancer therapy. *Nat Rev Cancer* 2008;8:193-204.
37. Lieberman HB. DNA damage repair and response proteins as targets for cancer therapy. *Curr Med Chem* 2008;15:360-367.
38. Kelley MR, Fishel ML. DNA repair proteins as molecular targets for cancer therapeutics. *Anticancer Agents Med Chem* 2008;8:417-425.
39. Robertson AG, Shih J, Yau C, et al. Integrative Analysis Identifies Four Molecular and Clinical Subsets in Uveal Melanoma. *Cancer Cell* 2017;32:204-220.e215.
40. Laurent C, Valet F, Planque N, et al. High PTP4A3 phosphatase expression correlates with metastatic risk in uveal melanoma patients. *Cancer Res* 2011;71:666-674.
41. Gangemi R, Mirisola V, Barisione G, et al. Mda-9/syntenin is expressed in uveal melanoma and correlates with metastatic progression. *PLoS One* 2012;7:e29989.
42. Gangemi R, Amaro A, Gino A, et al. ADAM10 correlates with uveal melanoma metastasis and promotes in vitro invasion. *Pigment Cell Melanoma Res* 2014;27:1138-1148.
43. Font RL CJ, Rao NA. Tumors of the uveal tract *Tumors of the Eye and Ocular Adnexa (AFIP Atlas of Tumor Pathology, Series 4, Fascicle 5)* Maryland: American Registry of Pathology; 2006:56-60.
44. Malignant melanoma of the uvea. In: Edge S BD, Compton CC, Fritz AG, Greene FL, Trotti A (ed), *AJCC Cancer Staging Manual.* New York: Springer; 2010:547-559.
45. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol* 2013;14:R36.

46. Chen PW, Murray TG, Uno T, Salgaller ML, Reddy R, Ksander BR. Expression of MAGE genes in ocular melanoma during progression from primary to metastatic disease. *Clin Exp Metastasis* 1997;15:509-518.
47. Amirouchene-Angelozzi N, Nemati F, Gentien D, et al. Establishment of novel cell lines recapitulating the genetic landscape of uveal melanoma and preclinical validation of mTOR as a therapeutic target. *Mol Oncol* 2014;8:1508-1520.
48. Luyten GP, Naus NC, Mooy CM, et al. Establishment and characterization of primary and metastatic uveal melanoma cell lines. *Int J Cancer* 1996;66:380-387.
49. Bengtsson H, Irizarry R, Carvalho B, Speed TP. Estimation and assessment of raw copy numbers at the single locus level. *Bioinformatics* 2008;24:759-767.
50. Bengtsson H SK, Bullard J, Hansen K. aroma.affymetrix: A generic framework in R for analyzing small to very large Affymetrix data sets in bounded memory, Tech Report #74. Department of Statistics, University of California, Berkeley, February 2008.
51. Bengtsson H, Wirapati P, Speed TP. A single-array preprocessing method for estimating full-resolution raw copy numbers from all Affymetrix genotyping arrays including GenomeWideSNP 5 & 6. *Bioinformatics* 2009;25:2149-2156.
52. Tschentscher F, Husing J, Holter T, et al. Tumor classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities. *Cancer Res* 2003;63:2578-2584.
53. van Gils W, Lodder EM, Mensink HW, et al. Gene expression profiling in uveal melanoma: two regions on 3p related to prognosis. *Invest Ophthalmol Vis Sci* 2008;49:4254-4262.
54. Goodwin JF, Kothari V, Drake JM, et al. DNA-PKcs-Mediated Transcriptional Regulation Drives Prostate Cancer Progression and Metastasis. *Cancer Cell* 2015;28:97-113.
55. Evert M, Frau M, Tomasi ML, et al. Deregulation of DNA-dependent protein kinase catalytic subunit contributes to human hepatocarcinogenesis development and has a putative prognostic value. *Br J Cancer* 2013;109:2654-2664.
56. Asnaghi L, Gezgin G, Tripathy A, et al. EMT-associated factors promote invasive properties of uveal melanoma cells. *Molecular Vision* 2015;21:919-929.
57. Friedberg EC. DNA damage and repair. *Nature* 2003;421:436-440.
58. Zhou BB, Elledge SJ. The DNA damage response: putting checkpoints in perspective. *Nature* 2000;408:433-439.
59. Shields CL, Ganguly A, Bianciotto CG, Turaka K, Tavallali A, Shields JA. Prognosis of uveal melanoma in 500 cases using genetic testing of fine-needle aspiration biopsy specimens. *Ophthalmology* 2011;118:396-401.
60. Jensen DE, Proctor M, Marquis ST, et al. BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances BRCA1-mediated cell growth suppression. *Oncogene* 1998;16:1097-1112.

61. Nishikawa H, Wu W, Koike A, et al. BRCA1-associated protein 1 interferes with BRCA1/BARD1 RING heterodimer activity. *Cancer Res* 2009;69:111-119.
62. Ismail IH, Davidson R, Gagne JP, Xu ZZ, Poirier GG, Hendzel MJ. Germline mutations in BAP1 impair its function in DNA double-strand break repair. *Cancer Res* 2014;74:4282-4294.
63. Cohn MA, Kee Y, Haas W, Gygi SP, D'Andrea AD. UAF1 is a subunit of multiple deubiquitinating enzyme complexes. *J Biol Chem* 2009;284:5343-5351.
64. Kennedy RD, D'Andrea AD. The Fanconi Anemia/BRCA pathway: new faces in the crowd. *Genes Dev* 2005;19:2925-2940.
65. Dunn J, Potter M, Rees A, Runger TM. Activation of the Fanconi anemia/BRCA pathway and recombination repair in the cellular response to solar ultraviolet light. *Cancer Res* 2006;66:11140-11147.
66. Nijman SM, Huang TT, Dirac AM, et al. The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. *Mol Cell* 2005;17:331-339.
67. Cohn MA, Kowal P, Yang K, et al. A UAF1-containing multisubunit protein complex regulates the Fanconi anemia pathway. *Mol Cell* 2007;28:786-797.
68. Park E, Kim JM, Primack B, et al. Inactivation of Uaf1 causes defective homologous recombination and early embryonic lethality in mice. *Mol Cell Biol* 2013;33:4360-4370.
69. Sands AT, Abuin A, Sanchez A, Conti CJ, Bradley A. High susceptibility to ultraviolet-induced carcinogenesis in mice lacking XPC. *Nature* 1995;377:162-165.
70. de Laat WL, Jaspers NG, Hoeijmakers JH. Molecular mechanism of nucleotide excision repair. *Genes Dev* 1999;13:768-785.
71. Sugasawa K, Ng JM, Masutani C, et al. Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. *Mol Cell* 1998;2:223-232.
72. Volker M, Mone MJ, Karmakar P, et al. Sequential assembly of the nucleotide excision repair factors in vivo. *Mol Cell* 2001;8:213-224.
73. Yang J, Xu Z, Li J, et al. XPC epigenetic silence coupled with p53 alteration has a significant impact on bladder cancer outcome. *J Urol* 2010;184:336-343.
74. Araki M, Masutani C, Takemura M, et al. Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair. *J Biol Chem* 2001;276:18665-18672.
75. Ramkumar HL, Brooks BP, Cao X, et al. Ophthalmic manifestations and histopathology of xeroderma pigmentosum: two clinicopathological cases and a review of the literature. *Surv Ophthalmol* 2011;56:348-361.
76. Sipley JD, Menninger JC, Hartley KO, Ward DC, Jackson SP, Anderson CW. Gene for the catalytic subunit of the human DNA-activated protein kinase maps to the site of the XRCC7 gene on chromosome 8. *Proc Natl Acad Sci U S A* 1995;92:7515-7519.

77. Jackson SP, Jeggo PA. DNA double-strand break repair and V(D)J recombination: involvement of DNA-PK. *Trends Biochem Sci* 1995;20:412-415.
78. Yoo S, Dynan WS. Geometry of a complex formed by double strand break repair proteins at a single DNA end: recruitment of DNA-PKcs induces inward translocation of Ku protein. *Nucleic Acids Res* 1999;27:4679-4686.
79. An J, Huang YC, Xu QZ, et al. DNA-PKcs plays a dominant role in the regulation of H2AX phosphorylation in response to DNA damage and cell cycle progression. *BMC Mol Biol* 2010;11:18.
80. Mehta A, Haber JE. Sources of DNA Double-Strand Breaks and Models of Recombinational DNA Repair. *Cold Spring Harbor Perspectives in Biology* 2014;6:a016428.
81. Mukherjee B, McEllin B, Camacho CV, et al. EGFRvIII and DNA double-strand break repair: a molecular mechanism for radioresistance in glioblastoma. *Cancer Res* 2009;69:4252-4259.
82. Shintani S, Mihara M, Li C, et al. Up-regulation of DNA-dependent protein kinase correlates with radiation resistance in oral squamous cell carcinoma. *Cancer Sci* 2003;94:894-900.
83. Li X, Tian J, Bo Q, et al. Targeting DNA-PKcs increased anticancer drug sensitivity by suppressing DNA damage repair in osteosarcoma cell line MG63. *Tumour Biol* 2015;36:9365-9372.
84. Cassoux N, Rodrigues MJ, Plancher C, et al. Genome-wide profiling is a clinically relevant and affordable prognostic test in posterior uveal melanoma. *Br J Ophthalmol* 2014;98:769-774.
85. Ehlers JP, Worley L, Onken MD, Harbour JW. DDEF1 is located in an amplified region of chromosome 8q and is overexpressed in uveal melanoma. *Clin Cancer Res* 2005;11:3609-3613.
86. Huang B, Shang ZF, Li B, et al. DNA-PKcs associates with PLK1 and is involved in proper chromosome segregation and cytokinesis. *J Cell Biochem* 2014;115:1077-1088.
87. Kotula E, Berthault N, Agrario C, et al. DNA-PKcs plays role in cancer metastasis through regulation of secreted proteins involved in migration and invasion. *Cell Cycle* 2015;14:1961-1972.
88. Kang GY, Pyun BJ, Seo HR, et al. Inhibition of Snail1-DNA-PKcs protein-protein interface sensitizes cancer cells and inhibits tumor metastasis. *J Biol Chem* 2013;288:32506-32516.
89. Velic D, Couturier AM, Ferreira MT, et al. DNA Damage Signalling and Repair Inhibitors: The Long-Sought-After Achilles' Heel of Cancer. *Biomolecules* 2015;5:3204-3259.

SUPPLEMENTS

Supplementary Table S1. Alphabetic list of all DNA repair genes (n=121, encoded by 178 probes) evaluated in our cohort. The expression of genes with a standard deviation > 0.3 (n=44, encoded by 49 probes) was compared between disomy 3 (n=24) and monosomy (n=40) tumors. Bonferroni correction was applied to the unrounded p-values. Significant corrected p-values and corresponding probes are in bold.

GENE	MEAN	SD	DISOMY 3 (n=24) Median (range)	MONOSOMY 3 (n=40) Median (range)	*P* VALUE	CORRECTED *P* VALUE
APEX1	9.76	0.58	10.2 (8.3-10.8)	9.6 (8.5-10.7)	0.001	0.07
APEX1a	10.69	0.42	11.0 (9.9-11.4)	10.5 (9.6-11.4)	<0.001	**0.004**
APEX2	7.87	0.21				
APITD1	Not in Illumina	Not in Illumina				
ATR	Not in Illumina	Not in Illumina				
ATRIP	7.33	0.22				
ATRIPa	7.12	0.21				
BAP1	7.52	0.53	8.0 (6.6-8.5)	7.4 (6.4-8.1)	<0.001	**<0.001**
BIVM-ERCC5	7.46	0.28				
BIVM-ERCC5a	7.82	0.31	7.9 (7.4-8.3)	7.8 (7.1-8.5)	0.05	1
BLM	6.46	0.11				
BRCA1	6.49	0.15				
BRCA1a	6.73	0.16				
BRCA2	Not in Illumina	Not in Illumina				
BRIP1	6.47	0.1				
C17orf70	9.1	0.39	9.0 (8.3-9.8)	9.2 (8.3-10.4)	0.02	0.84
C19orf40	7.06	0.19				
CCNH	7.54	0.34	7.4 (6.8-8.2)	7.6 (7.0-8.4)	0.008	0.38
CCNHa	7.66	0.22				
CDK7	8.89	0.39	8.8 (7.7-9.7)	8.9 (8.4-10.3)	0.21	1
CENPX	9.61	0.38	9.3 (8.9-10.3)	9.7 (9.0-10.6)	<0.001	**<0.001**
CETN2	10.02	0.38	10.2 (9.7-11.2)	9.9 (9.3-10.7)	<0.001	**0.002**
CUL4B	7.71	0.27				
CUL4Ba	6.53	0.17				
DCLRE1C	6.7	0.14				
DCLRE1Ca	7.28	0.32	7.2 (6.7-8.4)	7.3 (6.9-8.3)	0.31	1
DDB1	12.33	0.37	12.1 (11.3-13.0)	12.4 (11.7-13.0)	0.001	**0.04**
DDB1a	6.63	0.18				
DDB1b	6.9	0.18				
DDB1c	8.27	0.28				
DDB2	6.95	0.23				
DNTT	Not in Illumina	Not in Illumina				
EME1	6.54	0.1				

EME2	Not in Illumina	Not in Illumina				
ERCC1	9.52	0.27				
ERCC1a	7.38	0.3				
ERCC1b	9.74	0.29				
ERCC1c	6.53	0.11				
ERCC2	7.79	0.28				
ERCC3	7.98	0.17				
ERCC3a	6.6	0.11				
ERCC4	6.47	0.09				
ERCC6	6.48	0.11				
ERCC8	6.62	0.13				
ERCC8a	6.52	0.12				
EXO1	6.62	0.25				
EXO1a	6.39	0.13				
FAN1	8.71	0.28				
FAN1a	7.1	0.16				
FANCA	6.5	0.12				
FANCAa	6.52	0.11				
FANCAb	6.36	0.13				
FANCB	6.4	0.12				
FANCC	Not in Illumina	Not in Illumina				
FANCD2	6.74	0.2				
FANCE	7.59	0.36	7.7 (6.8-8.6)	7.4 (7.1-8.3)	0.003	0.13
FANCF	Not in Illumina	Not in Illumina				
FANCG	7.48	0.34	7.4 (7.0-8.5)	7.4 (6.9-8.4)	0.69	1
FANCI	6.59	0.18				
FANCL	6.42	0.14				
FANCLa	7.78	0.4	7.9 (7.1-8.6)	7.6 (6.9-8.9)	0.06	1
FANCM	Not in Illumina	Not in Illumina				
FEN1	7.09	0.19				
FEN1a	8.97	0.32	9.0 (8.4-9.7)	9.0 (8.1-9.6)	0.98	1
GTF2H1	6.91	0.19				
GTF2H1a	7.42	0.25				
GTF2H2	6.27	0.13				
GTF2H2B	7.13	0.32	7.0 (6.6-7.9)	7.2 (6.5-8.1)	0.37	1
GTF2H3	7.12	0.19				
GTF2H4	8.09	0.47	8.5 (6.9-9.4)	7.9 (7.2-9.3)	<0.001	**<0.001**
GTF2H5	10.19	0.43	10.1 (9.3-11.0)	10.3 (9.3-11.2)	0.01	0.62
LIG1	8.27	0.31	8.2 (7.6-8.7)	8.3 (7.8-9.0)	0.09	1
LIG3	6.85	0.12				
LIG3a	7.18	0.25				
LIG4	6.63	0.08				
LIG4a	6.47	0.11				
MBD4	8.26	0.46	8.4 (7.5-9.9)	8.1 (7.5-9.3)	0.33	1
MGMT	9.34	0.43	9.6 (8.7-10.3)	9.2 (8.3-10.1)		1

MLH1	7.94	0.33	8.2 (7.5-8.8)	7.8 (7.1-8.3)	<0.001	**<0.001**
MLH3	6.44	0.11				
MLH3a	6.63	0.15				
MNAT1	6.8	0.2				
MPG	6.61	0.09				
MPGa	6.31	0.12				
MRE11A	6.59	0.08				
MRE11Aa	6.74	0.17				
MSH2	6.46	0.12				
MSH2a	6.68	0.12				
MSH3	7.72	0.26				
MSH3a	13.39	0.65	13.3 (12.1-14.7)	13.5 (12.3-15.0)	0.13	1
MSH6	8.8	0.3				
MUS81	7.76	0.22				
MUTYH	6.61	0.12				
MUTYHa	7.85	0.38	7.9 (7.3-8.4)	7.9 (6.9-8.5)	0.39	1
MUTYHb	6.78	0.19				
NBN	8.1	0.42	7.9 (7.4-8.5)	8.2 (7.3-9.2)	0.005	0.24
NBNa	7.01	0.25				
NEIL1	6.58	0.11				
NEIL2	8.06	0.45	8.3 (7.4-8.8)	7.9 (6.9-9.1)	0.002	0.1
NEIL3	Not in Illumina	Not in Illumina				
NHEJ1	Not in Illumina	Not in Illumina				
NTHL1	7.76	0.31	7.8 (7.1-8.5)	7.7 (7.2-8.3)	0.01	0.64
OGG1	6.48	0.14				
OGG1a	6.94	0.2				
PALB2	7.21	0.19				
PARP2	7.43	0.22				
PARP2a	6.89	0.16				
PCNA	6.58	0.16				
PCNAa	8.53	0.51	8.3 (7.7-9.7)	8.6 (7.5-9.9)	0.002	0.12
PMS2	6.97	0.21				
PMS2a	6.44	0.15				
PMS2CL	6.79	0.14				
PMS2CLa	6.48	0.11				
POLB	9.72	0.63	9.9 (9.1-10.7)	9.7 (8.1-10.9)	0.06	1
POLD3	6.77	0.13				
POLE3	9.62	0.31	9.7 (8.8-10.3)	9.6 (9.1-10.3)	0.24	1
POLH	6.52	0.13				
POLHa	6.55	0.09				
POLI	Not in Illumina	Not in Illumina				
POLK	Not in Illumina	Not in Illumina				
POLL	6.89	0.2				

POLM	7.14	0.22				
POLN	6.53	0.22				
PRKDC	6.64	0.21				
PRKDCa	8.55	0.68	8.0 (7.3-8.6)	8.8 (7.8-10.2)	<0.001	**<0.001**
PRKDCb	6.64	0.15				
PRKDCc	6.54	0.12				
PRKDCd	6.56	0.1				
RAD23A	9.77	0.24				
RAD50	7.59	0.19				
RAD51	6.97	0.26				
RAD51a	6.81	0.13				
RAD51C	Not in Illumina	Not in Illumina				
RAD52	Not in Illumina	Not in Illumina				
RAD54B	6.52	0.1				
RAD54Ba	6.81	0.16				
RBX1	10.17	0.39	10.0 (9.4-10.8)	10.2 (9.6-11.1)	0.01	0.55
REV1	7.78	0.17				
REV1a	7.75	0.19				
REV3L	6.66	0.2				
RFC1	7.37	0.19				
RFC1a	8.66	0.28				
RMI1	6.84	0.14				
RMI1a	7.27	0.2				
RMI2	7.07	0.32	7.2 (6.7-7.7)	6.9 (6.5-7.7)	<0.001	**0.02**
RPA1	8.2	0.34	8.5 (7.7-8.9)	8.1 (7.4-8.9)	0.006	0.28
RPA1a	9.74	0.36	9.9 (8.7-10.4)	9.7 (8.9-10.5)	0.02	1
RPA1b	8.39	0.36	8.7 (7.7-9.2)	8.3 (7.4-8.9)	0.001	**0.04**
RPA2	9.95	0.38	10.1 (9.3-10.9)	10.0 (9.2-10.7)	0.22	1
RPA3	9	0.41	9.1 (8.5-10.1)	8.9 (8.0-9.9)	0.09	1
RPA4	6.38	0.13				
SEM1	11.32	0.24				
SEM1a	7.53	0.32	7.7 (7.3-8.4)	7.4 (6.8-8.0)	<0.001	**0.01**
SLX1A	Not in Illumina	Not in Illumina				
SLX1B	Not in Illumina	Not in Illumina				
SLX4	6.96	0.19				
SMUG1	9.04	0.23				
TDG	Not in Illumina	Not in Illumina				
TELO2	6.96	0.29				
TOP3A	7.39	0.36	7.3 (6.7-8.6)	7.4 (6.9-8.3)	0.22	1
TOP3Aa	6.78	0.15				
TOP3B	7.89	0.26				
UBE2T	7.32	0.37	7.3 (6.8-8.2)	7.3 (6.7-8.5)	0.49	1
UNG	6.31	0.12				
UNGa	9.6	0.41	9.6 (8.8-10.5)	9.7 (8.7-10.3)	0.53	1

UNGb	6.54	0.11				
USP1	7.57	0.29				
USP1a	6.48	0.12				
WDR48	7.82	0.36	8.2 (7.4-8.6)	7.6 (7.2-8.2)	<0.001	**<0.001**
XPA	6.99	0.14				
XPC	8.76	0.42	9.2 (8.3-9.7)	8.6 (8.0-9.3)	<0.001	**<0.001**
XRCC1	7.88	0.3	7.9 (7.3-8.7)	7.9 (7.3-8.5)	0.57	1
XRCC4	Not in Illumina	Not in Illumina				
XRCC5	8.94	0.32	9.0 (8.2-9.6)	9.0 (8.1-9.6)	0.95	1
XRCC6	8.5	0.31	8.3 (7.8-8.8)	8.6 (7.7-9.3)	0.005	0.26
XRCC6a	6.73	0.13				
XRCC6b	8.75	0.38	8.7 (7.9-9.2)	8.8 (7.9-10.0)	0.65	1
XRCC6c	10.61	0.37	10.5 (9.5-11.2)	10.7 (9.8-11.2)	0.02	0.87

Supplementary Table S2. Association of the expression of the 13 genes, which were significantly differentially expressed between disomy 3 and monosomy 3 tumors, with clinicopathologic parameters and survival. Significant p-values are in bold.

A: Genes which were highly expressed in monosomy 3 tumors:

CHARACTERISTIC		**GENE**	
	CENPX (17q25.3)	DDB1 (11q12)	PRKDC (8q11.21)
	PATHWAY		
	FA	NER	DSBR
LBD			
≤13 mm (n=34) *median(range)*	9.4 (9.0-10.6)	12.3 (11.3-13.0)	8.4 (7.5-9.8)
>13 mm (n=30) *median(range)*	9.7 (8.9-10.5)	12.3 (11.7-12.8)	8.7 (7.3-10.2)
P value *	**0.02**	0.93	0.11
Cell type			
Spindle (n=22) *median (range)*	9.5 (8.9-10.4)	12.3 (11.7-13.0)	8.2 (7.3-9.7)
Mixed/Epithelioid (n=42) *median(range)*	9.6 (9.0-10.6)	12.3 (11.3-13.0)	8.6 (7.5-10.2)
P value *	**0.04**	0.59	0.12
AJCC Stage			
Stage I (n=5) *median (range)*	9.3 (9.0-9.9)	12.6 (12.2-13.0)	8.2 (7.7-9.6)
Stage II (n=36) *median (range)*	9.5 (8.9-10.6)	12.3 (11.3-13.0)	8.4 (7.5-9.8)
Stage III (n=23) *median (range)*	9.7 (9.3-10.5)	12.3 (11.8-12.9)	8.7 (7.3-10.2)
P value †	**0.01**	0.1	0.19
Presence of metastases			
No (n=27) *median (range)*	9.3 (9.0-10.4)	12.3 (11.2-13.0)	8.1 (7.5-9.4)
Yes (n=37) *median (range)*	9.8 (8.9-10.6)	12.4 (11.7-13.0)	8.8 (7.3-10.2)
P value *	**<0.001**	0.3	**<0.001**

Survival analysis			
Expression lower than or equal to median	<=9.6	<=12.3	<=8.4
Expression higher than median	>9.6§	>12.3	>8.4§
P value ‡	**<0.001**	0.48	**0.001**

B: Genes which were lowly expessed in monosomy 3 tumors (part 1):

CHARACTERISTIC	**GENE**				
	APEX1 (14q11.2)	BAP1 (3p21.1)	CETN2 (Xq28)	GTF2H4 (6p21.33)	MLH1 (3p22.2)
	PATHWAY				
	BER	DSBR	NER	NER	MMR/FA
LBD					
≤13 mm (n=34) *median(range)*	10.7 (9.9-11.4)	7.7 (6.6-8.5)	10.0 (9.3-11.2)	8.0 (6.9-9.1)	8.0 (7.5-8.8)
>13 mm (n=30) *median(range)*	10.7 (9.6-11.4)	7.4 (6.4-8.5)	9.9 (9.3-10.9)	8.0 (7.2-9.4)	7.9 (7.1-8.6)
P value *	0.75	0.17	0.24	0.86	0.16
Cell type					
Spindle (n=22) *median (range)*	10.8 (9.6-11.4)	7.7 (6.6-8.5)	10.1 (9.6-10.9)	8.2 (7.2-9.4)	7.9 (7.1-8.8)
Mixed/Epithelioid (n=42) *median(range)*	10.6 (9.6-11.4)	7.6 (6.4-8.5)	10.0 (9.3-11.2)	8.0 (6.9-9.1)	7.9 (7.2-8.8)
P value *	0.13	0.15	0.1	0.12	0.97
AJCC Stage					
Stage I (n=5) *median (range)*	10.7 (10.4-11.0)	7.7 (7.5-8.1)	10.1 (9.3-11.2)	8.1 (7.9-8.5)	7.8 (7.6-8.4)
Stage II (n=36) *median (range)*	10.8 (9.7-11.4)	7.7 (6.4-8.5)	10.0 (9.6-10.7)	8.0 (6.9-9.4)	8.0 (7.5-8.8)
Stage III (n=23) *median (range)*	10.5 (9.6-11.3)	7.4 (6.6-8.5)	9.9 (9.3-10.9)	8.0 (7.5-9.1)	7.8 (7.1-8.6)
P value †	0.1	0.35	0.16	0.76	0.05
Presence of metastases					
No (n=27) *median (range)*	10.8 (9.9-11.4)	7.8 (6.6-8.5)	10.1 (9.7-11.2)	8.3 (6.9-9.3)	8.1 (7.5-8.8)

Yes (n=37) *median (range)*	10.6 (9.6-11.4)	7.4 (6.4-8.3)	9.9 (9.3-10.9)	7.9 (7.2-9.4)	7.9 (7.1-8.6)
P value *	0.11	**0.003**	**0.03**	**0.005**	**0.02**
Survival analysis					
Expression lower than or equal to median	<=10.7	<=7.6§	<=10	<=8§	<=7.9
Expression higher than median	>10.7	>7.6	>10	>8	>7.9
P value ‡	0.05	**0.001**	0.18	**0.001**	0.07

B: Genes which were lowly expessed in monosomy 3 tumors (part 2):

CHARACTERISTIC	**GENE**				
	RMI2 (16p13.13)	RPA1 (17p13.3)	SEM1 (7q21.3)	WDR48 (3p22.2)	XPC (3p25.1)
	PATHWAY				
	DSBR	DSBR/MMR/NER	DSBR	FA	NER
LBD					
≤13 mm (n=34) *median(range)*	7.09 (6.6-7.7)	8.4 (7.7-9.2)	7.5 (7.0-8.4)	8.0 (7.2-8.5)	8.9 (8.0-9.6)
>13 mm (n=30) *median(range)*	7.0 (6.5-7.7)	8.4 (7.4-8.8)	7.4 (6.8-8.0)	7.7 (7.2-8.6)	8.6 (8.0-9.7)
P value *	0.11	0.2	0.11	**0.01**	**0.004**
Cell type					
Spindle (n=22) *median (range)*	7.1 (6.5-7.7)	8.6 (7.4-9.2)	7.5 (6.8-8.0)	8.0 (7.3-8.6)	8.9 (8.0-9.7)
Mixed/Epithelioid (n=42) *median(range)*	6.9 (6.6-7.7)	8.4 (7.5-9.1)	7.5 (6.9-8.4)	7.7 (7.2-8.5)	8.7 (8.0-9.3)
P value *	0.15	0.06	0.09	**0.007**	**0.03**
AJCC Stage					
Stage I (n=5) *median (range)*	7.1 (6.9-7.6)	8.7 (7.9-9.1)	7.6 (7.1-8.2)	7.9 (7.5-8.5)	8.8 (8.6-9.2)
Stage II (n=36) *median (range)*	7.0 (6.6-7.7)	8.5 (7.7-9.2)	7.5 (7.0-8.4)	8.1 (7.3-8.6)	8.8 (8.0-9.7)
Stage III (n=23) *median (range)*	7.1 (6.5-7.7)	8.3 (7.4-8.8)	7.4 (6.8-8.0)	7.7 (7.2-8.3)	8.6 (8.0-9.3)
P value †	0.44	**0.03**	0.27	0.08	0.14

Presence of metastases					
No (n=27) *median (range)*	7.1 (6.7-7.7)	8.4 (7.7-9.2)	7.6 (7.3-8.4)	8.1 (7.2-8.6)	9.0 (8.0-9.6)
Yes (n=37) *median (range)*	6.9 (6.5-7.7)	8.4 (7.4-8.9)	7.4 (6.8-8.01)	7.7 (7.2-8.3)	8.6 (8.0-9.7)
P value *	**0.003**	0.09	**0.006**	**<0.001**	**0.006**
Survival analysis					
Expression lower than or equal to median	<=7.1§	<=8.4	<=7.5§	<=7.8§	<=8.7§
Expression higher than median	>7.1	>8.4	>7.5	>7.8	>8.7
P value ‡	**0.02**	0.41	**0.006**	**<0.001**	**0.005**

*Symbols: * Mann-Whitney U test, † Kruskal-Wallis test, ‡ Log-rank rest, § worse survival*