420 Molecular mechanisms in uveal melanoma
Wednesday, May 10, 2017 8:30 AM–10:15 AM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 3954–3972/A0241–A0259
Organizing Section: Anatomy and Pathology/Oncology

Program Number: 3954 **Poster Board Number:** A0241
Presentation Time: 8:30 AM–10:15 AM
Characterization and prognostic significance and of small epithelioid cell populations in uveal melanoma
Pablo Zoroquiain, Jose Joao Mansure, Ciro Garcia, Maria Antonia Saornil, William April, Miguel N. Burnier. Pathology, McGill University, Montreal, QC, Canada.
Purpose: Uveal melanoma (UM) is a malignant neoplasia that arises from uveal melanocytes. Despite the availability of various treatment modalities, approximately 40% of patients develop metastases, 90% of which will succumb to their disease. UM is composed of two main type of cells: spindle and epithelioid. However, there is a subset of neoplastic small epithelioid cells that are located predominately in the infiltrative tumor margins or surrounding blood vessels. The aim of this study is to characterize and evaluate the correlation between the presence of these small epithelioid cells and clinical outcome.
Methods: The clinical-pathological features of 70 UM patients were evaluated. The presence of small epithelioid cells was quantified based on percentage of tumor volume. Furthermore, in a subset of cases (n=6) the small epithelioid cells were characterized using melanocytic markers (HMB-45, Melan A and SOX-10), stem cell markers (CD133, CD24 and CD38) and T cell lymphocytes (CD3). Univariate and multivariate analyses were conducted to evaluate survival. Clinical follow up was available for all patients (mean 148.6 months; SEM 15.1)
Results: Results: The ratio of small epithelioid cell components of all 70 tumors ranged from 0% to 30% (median, 1%). Thirty-nine tumors (55.7%) had areas with small epithelioid cells. Univariate analysis showed that mixed versus spindle cell (P=0.006), higher lymphocytic infiltration (P=0.04), macrophage infiltration (P=0.02), ciliary body involvement (P=0.02) and >5% of small epithelioid cell component (P<0.0001) had a significant negative impact on metastasis-free survival. Small epithelioid cell component >5% was present in 24 cases (34.3%). Of these, three were classified as spindle and 21 were mixed. Multivariate analysis revealed that a >5% small cell component was the most significant morphological adverse prognostic factor (P<0.001, HR=4.98). Moreover, the small epithelioid cells were negative for HMB45, focally and weakly positive for MELAN A and SOX10, and were negative for stem cell markers and CD3.
Conclusions: A high small epithelioid cell component is a strong negative prognostic indicator in patients with UM. This feature might be useful to prognosticate enucleated patients when molecular studies are not available. Further characterization may lead to new therapeutic targets and a better understanding of the metastatic process in UM.
Commercial Relationships: Pablo Zoroquiain; **Jose Joao Mansure**, None; **Ciro Garcia**, None; **Maria Antonia Saornil**, None; **William April**, None; **Miguel N. Burnier**, None

Program Number: 3955 **Poster Board Number:** A0242
Presentation Time: 8:30 AM–10:15 AM
Expression of anaplastic lymphoma kinase in uveal melanoma
Jacqueline Coblentz, Patrick T. Logan, Ana Beatriz T. Dias, Evangelina Esposito, Jose Joao Mansure, Miguel N. Burnier. Ocular Pathology, McGill University, Westmount, QC, Canada.
Purpose: Uveal melanoma (UM) is a disease that affects approximately five people per million in the United States. This disease metastasizes predominantly to the liver, and treatment options following clinical detection of these sequelae are limited. Anaplastic lymphoma kinase (ALK) is a transmembrane receptor kinase that belongs to the insulin receptor superfamily and has previously been shown to play a role in cell proliferation, migration and invasion in several malignant tumors, including lymphoma, non-small cell lung cancer, and skin melanoma. The purpose of this study was to determine the expression of anaplastic lymphoma kinase (ALK) in UM in order to evaluate its potential utility as a therapeutic target.
Methods: Eighty formalin-fixed paraffin-embedded enucleated eyes of patients with UM, 11 eyes from a rabbit model of UM, and 11 pulmonary metastasis from this model were collected from the Henry C. Witelson Ocular Pathology Laboratory, McGill University, Montreal, Canada. All samples were stained for ALK using a fully-automated immunohistochemical procedure. The presence of ALK was confirmed for any tumors that showed positive expression. Human UM cases were classified according to cell type: spindle or mixed (predominantly epithelioid). Differences in ALK positivity according to cell type were determined using Pearson's Chi-square test.
Results: In human UM specimens, ALK was positive in 2 spindle cell type cases (2.5%) and in 13 (16.25%) mixed cell type cases. The difference in ALK expression between cell types was statistically significant (P=0.02). Regarding the animal model, in which all animals were inoculated with mixed cell type tumors, all cases (100%) were positive for ALK in both primary and pulmonary lesions.
Conclusions: ALK is expressed in the minority of human eyes with UM, with statistically greater expression in the more aggressive, mixed cell type. ALK is also expressed in all primary UMs and pulmonary metastasis in an established, highly metastatic animal model of UM. Therefore, our data suggest that ALK expression is more prevalent in aggressive tumors. To the best of our knowledge, this is the first demonstration of ALK's potential as a therapeutic target in human UM, and in particular aggressive tumors.
Commercial Relationships: Jacqueline Coblentz, None; **Patrick T. Logan**, None; **Ana Beatriz T. Dias**, None; **Evangelina Esposito**, None; **Jose Joao Mansure**, None; **Miguel N. Burnier**, None

Program Number: 3956 **Poster Board Number:** A0243
Presentation Time: 8:30 AM–10:15 AM
The effect of SIRT1 inhibition by EX527 on uveal melanoma cells
Debra-Meghan Sanft[1, 2], Sultan Aldrees[2], Sabrina Bergeron[2], Jade Lasiste[2], Denise Miyamoto[2], Miguel N. Burnier[2, 1].
[1]Ophthalmology, McGill University, Montreal, QC, Canada; [2]MUHC-McGill University Ocular Pathology Laboratory, Montreal, QC, Canada.
Purpose: SIRT1 expression has previously been documented in a variety of malignancies, including uveal melanoma (UM), and is believed to inactivate proteins involved in tumor suppression and DNA repair. EX527 is a specific SIRT1 inhibitor that has been shown to decrease cutaneous melanoma proliferation in culture. Herein, we investigated the effects of EX527 on the proliferation and migration of a human uveal melanoma cell line.
Methods: The OCM-1 cell line was used to test the effect of EX527 on its proliferative and migratory abilities. For the proliferation assay, the cells were seeded in triplicates at a concentration of 5000 cells/well in a 96-well plate. The cells were serum starved for 24 hours and then the drug was added in three different concentrations: 10, 25, and 50 μM for 48 hours. At the end of the

experiment, the number of cells in each well were estimated using a colorimetric cellular proliferation. To test the effects of EX527 on migration, the experiment with the same drug concentrations was repeated using a cell migration assay as per manufacturer instructions.
Results: Inhibiting SIRT1 using EX527 decreased the proliferation rate of the tested cell line in a dose dependent manner. Compared to the control, 10 μM of EX527 did not significantly decrease proliferation (P=0.07). However, higher doses (25 and 50 μM) significantly inhibited proliferation (P<0.01). Similarly, EX527 decreased the number of migrating cells in a dose dependent manner. Compared to 10 μM, 25 μM of EX527 did not significantly decrease migration (P=0.22). However, the highest concentration (50 μM) significantly decreased migration compared to 10 and 25 μM (P<0.01). The control group had the highest migration.
Conclusions: To the best of our knowledge, this is the first study to evaluate the effects of a specific SIRT1 inhibitor; EX527, on UM cells in vitro. At physiologically relevant doses, EX527 inhibited both UM cell migration and proliferation, making it a suitable candidate for future studies. The effects of this drug should be explored in an animal model of this disease to determine administration routes, doses, and potential side effects.
Commercial Relationships: Debra-Meghan Sanft, None; **Sultan Aldrees**, None; **Sabrina Bergeron**, None; **Jade Lasiste**, None; **Denise Miyamoto**, None; **Miguel N. Burnier**, None

Program Number: 3957 **Poster Board Number:** A0244
Presentation Time: 8:30 AM–10:15 AM
Searching for genes predisposing to bilateral Uveal Melanoma (bUM) by exome sequencing of three unrelated cases
Antonio Pineiro[1], Paula Silva[2], Lourdes Loidi[2], Maria Santiago-Varela[1], Manuel F. Bande[1], Maria Pardo[3], María José Blanco[1]. [1]Unidad de Retina Quirúrgica y Tumores Intraoculares del Adulto, Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain; [2]Fundación Xenómica, Santiago de Compostela, Spain; [3]Grupo de Obesidómica, Fundación Ramón Dominguez, Santiago de Compostela, Spain.
Purpose: Uveal melanoma (UM) is the most common primary intraocular tumor in adults, with an annual incidence of approximately 6 cases per million people. Bilateral Uveal Melanoma (bUM) is rarer than UM with about 50 well-documented cases published since 1930. Considering its extremely low incidence, we hypothesize that it is very unlikely to find patients suffering from bUM in the same geographical region unless some shared genetic background predisposes to it.
The aim of this study is to identify germline causing and/or predisposing genetic variants in 3 patients diagnosed of bUM.
Methods: We perform a retrospective study in 3 unrelated Caucasian patients (2 men and 1 woman) with ages of 80, 56 and 96 year-old, diagnosed and treated of bUM. Complete ophthalmic examination and ocular ultrasonographic study were carried out in both eyes of each patient. A complete screening of the systemic disease was performed in all of them. All patients signed a special informed consent to start a full genomic study, both in the pathological pieces from two eyes enucleated from two patients and blood samples. Exome sequencing was performed in DNA obtained from blood samples of the 3 bUM patients (IonTorrentTM PGM® System platform, Thermo Fisher). Data obtained from Exome sequencing were screened for shared germline rare genetic variants in the main genes related to UM (35 genes) by the 3 bUM cases. In addition, some common genetic background in non-coding flanking regions of these genes was investigated.
Results: We found no rare variants shared by the 3 cases of bUM in the major genes related to UM. These results suggested an independent genetic way of developing bUM. Even tough, we found a shared genetic profile in some genes related to UM as *GNAQ, CDKN2A, IGF1R, TP53, CDH5, VEGFA, HGF* and *CXCR4.*Most of the shared variants have high population frequencies and are situated in non-coding flanking regions. However, it is not possible to rule out that this genetic profile is to some increase risk of developing bUM.
Conclusions: The cause of developing a bUM is apparently not linked to the existence of rare genetic variants in common UM genes. Further studies should be made to better confirm or refute the existence of a genetic profile that increase the risk of developing bUM. Next step of the study will be focused in searching for new predisposing genes for bUM.
Commercial Relationships: Antonio Pineiro, None; **Paula Silva**, None; **Lourdes Loidi**, None; **Maria Santiago-Varela**, None; **Manuel F. Bande**, None; **Maria Pardo**, None; **María José Blanco**, None

Program Number: 3958 **Poster Board Number:** A0245
Presentation Time: 8:30 AM–10:15 AM
MicroRNA-1 Regulates GNAQ Expression in Uveal Melanoma Cells
Dongsheng Yan[1], Lihua Wang[1], JIao Wang[1], Xiaoyan Chen[1], Dan-Ning Hu[2]. [1]School of Ophthalmology and Optometry, Wenzhou Medical University, Wenzhou, China; [2]The New York Eye and Ear Infirmary, New York Medical College, New York, NY.
Purpose: GNAQ is a heterotrimeric G protein alpha subunit, which when mutated can act as an oncogene. Such a change can make it a crucial contributor to the development of uveal melanoma. There are numerous studies showing that aberrant microRNA (miRNA) expression patterns contribute to tumorigenesis. However, whether GNAQ can be modulated by miRNA, remains elusive. In a previous study, we reported that miR-1 can inhibit uveal melanoma cell proliferation and migration through the c-Met pathway. Here, we determined whether miR-1 regulates GNAQ expression in uveal melanoma cells.
Methods: Bioinformatics predicted miR-1 gene targets. Luciferase reporter assay validated putative target identity. MiR-1 or GNAQ specific siRNA was transfected into uveal melanoma cells by Lipofectamine RNAiMAX reagent. Western blot analysis was carried out to detect GNAQ expression in uveal melanoma cells. Cell proliferation and migration were measured by MTS and transwell assay, respectively.
Results: GNAQ was identified as a bona fide target of miR-1. Ectopic miR-1 downregulated GNAQ expression in uveal melanoma cells. Downregulation of GNAQ significantly inhibited uveal melanoma cell proliferation and migration.
Conclusions: Our results demonstrated that GNAQ is a miR-1 gene target in uveal melanoma cells. MiR-1 may be a promising drug target to treat uveal melanoma due to its pleiotropic regulation of multiple oncogenes.
Commercial Relationships: Dongsheng Yan, None; **Lihua Wang**, None; **JIao Wang**, None; **Xiaoyan Chen**, None; **Dan-Ning Hu**, None
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Presentation Time: 8:30 AM–10:15 AM
Microenvironmental Regulation of the Liver Inflammasomes in Uveal Melanoma Favors Metastatic Growth
Vanessa M. Morales[1, 2], Zachary K. Goldsmith[1], Neel Patel[1], Benjamin A. King[1], Hans E. Grossniklaus[3], Matthew W. Wilson[1, 4]. [1]Ophthalmology, Hamilton Eye Institute, University of Tennessee Health Science Center, Memphis, TN; [2]Microbiology, Immunology and Biochemistry, UTHSC, Memphis, TN; [3]Ophthalmology, Emory University, Atlanta, GA; [4]Surgery, St. Jude Childrens Research Hospital, Memphis, TN.
Purpose: Many challenges remain in our understanding of the development of metastasis. Uveal melanoma disseminates hematogenously to the liver. The events in the microenvironment leading to immune cell activation for tumor clearance are not fully understood. The recently described inflammasomes are signaling scaffolds that trigger innate immune activation promoting pyroptosis, a recently described type of programmed cell death by activation of caspase-1. In this study we investigated the presence of inflammasomes in the metastatic liver in an orthotopic xenograft uveal melanoma animal model and a patient *post mortem*.
Methods: We received approval for the Institutional Review Board at the University of Tennessee Health Science Center to perform analyses on *post mortem* tissue of uveal melanoma patients and compared them to healthy livers. Liver samples of an orthotopic xenograft model were also examined. We performed Western blot analyses on 4-described inflammasomes: NLRP1, NLRP2, NLRP3, and NLRP12, in addition to caspase-1 analysis.
Results: Western blot results show absence of most known inflammasomes in the metastatic uveal melanoma liver compared to healthy liver. Multiple areas of the metastatic liver were examined and found similar results. We investigated expression of caspase-1 in these samples and found presence only in healthy liver samples.
Conclusions: Our work suggests the liver inflammasommes may contribute to clearance of metastasis in the liver. Further work is needed to understand the components of the liver inflammasomes and define factors, which may serve as drug targets to reduce metastasis.
Commercial Relationships: Vanessa M. Morales, None; **Zachary K. Goldsmith**, None; **Neel Patel**, None; **Benjamin A. King**, None; **Hans E. Grossniklaus**, None; **Matthew W. Wilson**, None
Support: Research to Prevent Blindness, West Cancer Center

Program Number: 3960 **Poster Board Number:** A0247
Presentation Time: 8:30 AM–10:15 AM
PKC-delta as a Survival Regulator in Uveal Melanoma
Bradley Gao[1], Mercy Kibe[1], Kelley Yuan[1], Hans E. Grossniklaus[2], Matthew W. Wilson[1, 3], Vanessa M. Morales[1, 4]. [1]Ophthalmology, Hamilton Eye Institute, UTHSC, Memphis, TN; [2]Ophthalmology, Emory University, Memphis, TN; [3]Surgery, St. Jude Childrens Research Institute, Memphis, TN; [4]Microbiology, Immunology and Biochemistry, UTHSC, Memphis, TN.
Purpose: Our laboratory focuses on uveal melanoma (UM), the most common primary intraocular malignancy in adults. Previously, we found the expression of phosphorylated PKC-delta (pPKC-d) higher in eyes with UM compared to healthy ones. Inhibition of paxillin, a cytoskeletal adaptor protein, reduced the phosphorylation status of PKC-d in UM cell lines. While paxillin inhibition in the past have shown promise in the treatment of primary and metastatic cancer, paxillin is an ubiquitous protein, which makes it difficult to translate into a targeted drug therapy. Because of the co-localization of PKC-d with paxillin, we investigated the effects of pharmacological inhibition and RNA interference of PKC-d in UM cell lines.
Methods: We performed RNA interference experiments in Mel270 (primary cell line) and OMM2.5 (metastatic cell line) targeting *PKCD*. As for our controls, we used scrambled siRNA. Transduced cells were examined for morphological changes, mRNA expression and Western blot. As a pharmacological inhibitor, we used rottlerin, a high specific PKC-d inhibitor.
Results: Silencing of *PKCD* was confirmed by qPCR and by Western blot analyses. In the cell culture, we observed an increase in apoptosis in the treated group when compared to our controls. Western Blot analysis exhibited a significant down-regulation of AKT and a significant increase in Caspase-3. Similar results were achieved when rottlerin was used.
Conclusions: Our results suggest that direct targeting of PKC-d have similar results to paxillin inhibition. We expect this targeted therapy could have less undesirable clinical effects compared to the inhibition of an ubiquitous molecule such as paxillin.
Commercial Relationships: Bradley Gao, None; **Mercy Kibe**, None; **Kelley Yuan**, None; **Hans E. Grossniklaus**, None; **Matthew W. Wilson**, None; **Vanessa M. Morales**, None
Support: Research to Prevent Blindness, West Cancer Center Medical Student Fellowship, West Cancer Center Research Grant

Program Number: 3961 **Poster Board Number:** A0248
Presentation Time: 8:30 AM–10:15 AM
Evaluation of Germline Variants in the Promotor Region of Macrophage Migration Inhibitory Factor (MIF) in Uveal Melanoma Patients
Stanley Park, Benjamin Romney, Khara Walker, Robert Pilarski, Lynn Schoenfield, Frederick Davidorf, Colleen M. Cebulla, Mohamed H. Abdel-Rahman. Ophthalmology, Ohio State, Columbus, OH.
Purpose: Two polymorphisms, rs755622 (previously reported as -173G>C) and rs5844572 (previously reported as -794 $(CATT)_{5-8}$), in the promoter region of Macrophage Migration Inhibitory Factor *(MIF)* gene influence the basal and/or induced transcriptional activity of *MIF* and have been linked to several inflammatory diseases and outcome of several cancers. We investigated the potential role of these polymorphisms in the UM.
Methods: Germline DNA was extracted from mononuclear cells at the OSU Human Cancer Genetic sample bank according to published protocol using a simple salting out procedure. A total of 163 patients were included in the study. The average age of the patients was 58 years old (range 18-86), 87 (53.4%) were males and 76 (46.6%) were females. The rs755622 variant was studied using Taqman real-time PCR and the rs5844572 using fluorescence-based genotyping. Allele frequencies were determined for both polymorphisms in the patient cohort. The allele frequency in the general population was assessed in 102 healthy adults from the same geographical location. The levels of MIF expression in the UM tumors was studied in a subset of primary tumors, as well as, tumor cell lines and normal cadaver choroid using Western blot.
Results: The rs755622 variant was successfully assessed in 151 patients while the rs5844572 variant was assessed in all patients. The allele frequencies of the rs755622 variant were C/C (4.6%), G/C (26.5%) and G/G (68.9%). The allele frequencies of the rs5844572 variant were as follows- 5/5 (9.8%), 5/6 (35%), 5/7 (5.5%), 5/8 (0%), 6/6 (33.1%), 6/7 (14.7%), 6/8 (0%), 7/7 (1.8%), 7/8 (0%). The allele frequency for the general population showed no statistical difference from the UM cohort (data not shown). Finally, the MIF protein expression was higher in the tumors compared to the controls.
Conclusions: The UM sample population appears to follow a similar allelic distribution as the control population for these polymorphisms and thus does not appear to be associated with risk of UM.

However, we identified higher MIF expression in the tumors than the controls with a limited tissue cohort. We are expanding the study to include additional tumors samples. The correlation between the polymorphisms and patient survival as well as tumor MIF expression, tumor somatic genetic alterations, tumor inflammatory cellular infiltrate and histology will be presented.
Commercial Relationships: Stanley Park, None; **Benjamin Romney**, None; **Khara Walker**, None; **Robert Pilarski**, None; **Lynn Schoenfield**, None; **Frederick Davidorf**, None; **Colleen M. Cebulla**, None; **Mohamed H. Abdel-Rahman**, None
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Program Number: 3962 **Poster Board Number:** A0249
Presentation Time: 8:30 AM–10:15 AM
Comparison of FISH and Cytogenomic Microarray Analysis for Detection of Chromosomal Abnormalities in Uveal Melanoma
Faris Hashem[1, 2], Nosaibah Hariri[3, 4], James Solomon[3], John Thorson[3], Marie Dell'Aquila[5, 6], Bobby Korn[1], Don Kikkawa[1], Michael H. Goldbaum[1], Jonathan Lin[1, 3]. [1]Ophthalmology, Shiley Eye Institute, University of California San Diego, La Jolla, CA; [2]Ophthalmology, University of Tabuk, Tabuk, Saudi Arabia; [3]Pathology, University of California San Diego, La Jolla, CA; [4]Pathology, University of Tabuk, Tabuk, Saudi Arabia; [5]Medical Genetics, Medicine, University of California San Diego, La Jolla, CA; [6]Cytogenetics/Cytogenomics Laboratory, University of California San Diego, La Jolla, CA.
Purpose: Uveal melanoma has high tendency for hematogenous metastasis to the liver. Fluorescence in situ hybridization (FISH) testing is routinely done to detect monosomy 3 and predict the likelihood of metastasis. We compared the sensitivity and specificity of FISH testing with cytogenomic microarray single nucleotide polymorphism (SNP) analysis in detecting chromosomal abnormalities in uveal melanoma specimens.
Methods: Uveal melanoma enucleation specimens were collected from 2014 to 2016. Five patients were included, 4 were male and 1 was female. The age range was from 40 to 80. FISH testing was performed on unstained sections from 4 enucleations. OncoScan cytogenomic microarray SNP analysis was performed on 5 cases, 4 of them from formalin-fixed enucleation tissue extracted, and in 1 case the material was taken from fine needle aspiration of vitreal fluid. FISH and OncoScan results were compared. Formalin fixed, paraffin-embedded tissue blocks from the enucleation specimens were also examined by hematoxylin and eosin (H&E) for histopathologic tumor staging and grading.
Results: Histologically, 3 uveal melanoma cases had mixed spindle and epithelioid type morphology, 1 case was spindle type, and 1 case was epithelioid type. FISH testing showed that 3 cases had <20% of monosomy 3 in tumor cells (normal), and in 1 case, 74.5% of tumor cells showed monosomy 3. OncoScan results revealed abnormality in all 5 cases. One case showed loss of heterozygosity (LOH) involving the BAP1 locus on chromosome 3. The second case showed complete LOH for BAP1, loss of Y chromosome and 6q, gain of 4p (FGFR3, WHSC1, SLC34A2, RHOH and PHOX2B), 6p, 8q, 2p24.3-p24.3 (MYCN) and 9p. The third case had loss 6q, 16q, 17p, 17q and 21q, gain of 6p, 16p, 17q (proximal segment), most of 21q and gain of 4q12-q22.1 (PDGFRA, KIT), 6p22.1-p21.1 (CCND3), 8q (MYC). The fourth case had loss of X chromosome, homozygous deletion 22q11.23 (GSTT1), and gain of 6p. The fifth case showed loss of Y, gain of 9, loss of distal 14q, and low-level loss of 16q/proximal 16p.
Conclusions: Our findings suggest that OncoScan microarray analysis is more sensitive than FISH test in detecting chromosomal abnormalities in uveal melanomas particularly small deletions and duplications. Interestingly, OncoScan can also be performed on small vitreal samples.
Commercial Relationships: Faris Hashem, None; **Nosaibah Hariri**, None; **James Solomon**, None; **John Thorson**, None; **Marie Dell'Aquila**, None; **Bobby Korn**, None; **Don Kikkawa**, None; **Michael H. Goldbaum**, None; **Jonathan Lin**, None
Support: UCSD Vision Core Research Grant P30EY022589

Program Number: 3963 **Poster Board Number:** A0250
Presentation Time: 8:30 AM–10:15 AM
Differential expression of DNA repair genes in prognostically-good and bad Uveal Melanoma
Mehmet Dogrusoz[1], Andrea Ruschel Trasel[2], Sake I. van Pelt[1], Sjoerd G. van Duinen[3], Pieter A. van der Velden[1], Gregorius P. Luyten[1], Martine J. Jager[1]. [1]Ophthalmology, LUMC, The Hague, Netherlands; [2]Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; [3]Pathology, LUMC, Leiden, Netherlands.
Purpose: To determine the expression of DNA repair genes in uveal melanoma (UM) and to test whether certain DNA repair genes are differentially expressed between prognostically-good and bad tumors
Methods: A genome-wide gene expression analysis was performed in 64 UMs that were primarily enucleated in the LUMC (Leiden, The Netherlands) between 1999 and 2008. Expression of 117 DNA repair genes was analyzed, and genes with variable expression were selected for further analysis. Expression was compared to histopathological data and survival for genes that showed a significantly differential expression between disomy 3 (D3) and monosomy 3 (M3) tumors. Bonferroni correction was applied for multiple comparisons. Results were validated by analyzing the expression of these genes in relation to survival in a publicly available set of 110 UMs from the University of Genoa (Italy) and Curie Institute, Paris (France).
Results: We identified 44 DNA repair genes that showed a sufficient level (cut-off standard deviation: ≥ 0.31) of variation in expression. A high expression of PRKDC (p=0.002) and STRA13 (p<0.001) was associated with poor survival in the LUMC set, as were a low expression of XPC (p<0.001), GTF2H4 (p=0.001), BAP1 (p=0.002), SHFM1 (p=0.006), and WDR48 (p<0.001). The associations with survival for PRKDC, XPC, BAP1, and WDR48 (Figure 1) were confirmed in the validation set. The expression values of these genes corresponded positively with the copy number of the respective chromosomes harboring these genes (PRKDC: chromosome 8q; XPC, BAP1 and WDR48: chromosome 3) (all p=0.001). A low expression of XPC and WDR48 was related to a large tumor diameter (p=0.004 and p=0.01, respectively) and a mixed/epithelioid cell type (p=0.03 and p=0.007, respectively).
Conclusions: Certain DNA repair genes are differentially expressed between prognostically-good and bad UMs. An increased understanding of the role of DNA repair genes in UM may result in the identification of new targets of therapy.

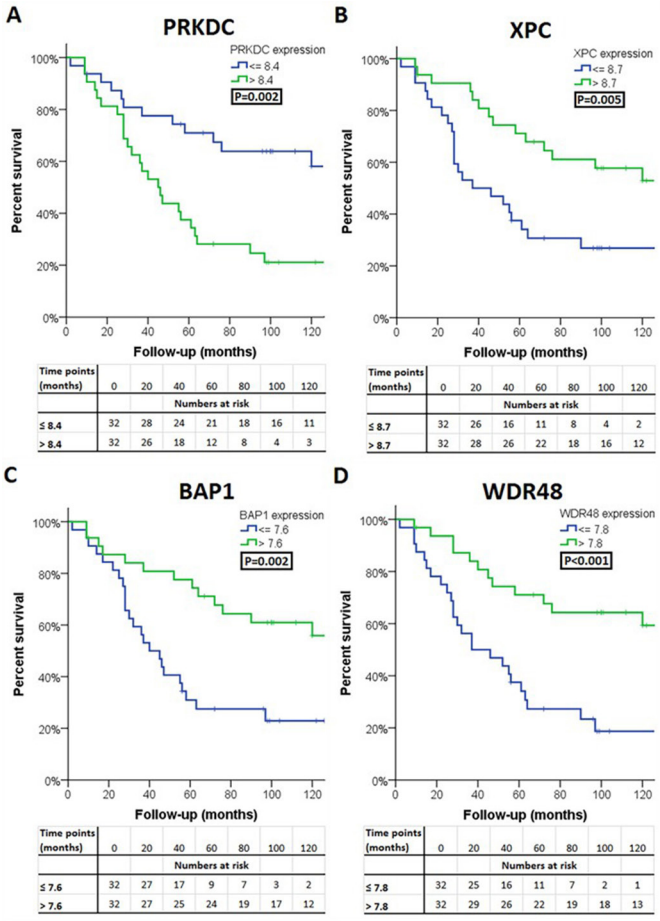

Figure 1. Survival curves of DNA repair genes that were significantly associated with survival in both sets. *Only the curves for the LUMC cohort are shown. Tumors were divided in two groups by the median expression value.*

Commercial Relationships: Mehmet Dogrusoz, None; **Andrea Ruschel Trasel**, None; **Sake I. van Pelt**, None; **Sjoerd G. van Duinen**, None; **Pieter A. van der Velden**, None; **Gregorius P. Luyten**, None; **Martine J. Jager**, None
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Program Number: 3964 **Poster Board Number:** A0251
Presentation Time: 8:30 AM–10:15 AM
Metformin inhibits survival, migration, and vascular endothelial growth factor production in uveal melanoma cells
Bruna Duarte Moron de Andrade, Jade Lasiste, Denise Miyamoto, Catherine Pancini Rezende, Christina Mastromonaco, Miguel N. Burnier. MUHC - McGill University Ocular Pathology Laboratory, Montreal, QC, Canada.
Purpose: Uveal melanoma is the most common intraocular malignancy in adults. Despite advances in treatment, 50% of patients develop metastasis, 90% of which succumb to their disease. Metformin, a biguanide drug used primarily for the treatment of diabetes, has an excellent safety profile. Metformin has also been shown to reduce proliferation, metastasis and angiogenesis in a variety of tumor cells, including cutaneous melanoma. However, it has not yet been tested on uveal melanoma. The objective of this study is to determine the effect of metformin on: (1) survival, (2) migration and (3) production of the angiogenesis precursor vascular endothelial growth factor (VEGF) in the uveal melanoma cell line OCM-1.
Methods: Metformin treatment concentrations ranging from 0-100 mM were used. The cell counting kit-8 (CCK-8) viability assay was utilized to test the cytotoxicity of metformin. The scratch assay, with subsequent Image analysis, was performed to assess the effect of metformin on migration. To promote angiogenesis and test the effect of metformin on VEGF production, cells were grown in a hypoxic (1% oxygen) chamber. Secreted VEGF was quantitated using a sandwich enzyme-linked immunosorbent assay (ELISA). Statistical analysis was done using Kruskal-Wallis with Dunn's multiple comparison test for data on cytotoxicity and angiogenesis, while analysis of variance (ANOVA) with Tukey post-hoc test was used for data on migration.
Results: Metformin was lethal to half and all of the cells at 30 mM and 70 mM (LC50 and LC100, respectively). There was a significant decrease in survival ($P<0.05$) at 30 mM. To avoid bias due to cytotoxicity, all subsequent experiments were performed with 0-20mM metformin. Metformin significantly inhibited cell migration at 5mM metformin ($P<0.05$). VEGF production also decreased with the lowest dose of metformin (0.1 mM) and significantly at 5 mM ($P<0.05$).
Conclusions: Metformin inhibits survival, migration and production of VEGF in human uveal melanoma cells and therefore has potential as an adjunct to therapy. Studies on its mechanism of action and toxicity to proximal eye tissues should be performed. Effectiveness in *in vivo* models must be tested to determine the ideal dose, route and timing of administration.
Commercial Relationships: Bruna Duarte Moron de Andrade, None; **Jade Lasiste**, None; **Denise Miyamoto**, None; **Catherine Pancini Rezende**, None; **Christina Mastromonaco**, None; **Miguel N. Burnier**, None

Program Number: 3965 **Poster Board Number:** A0252
Presentation Time: 8:30 AM–10:15 AM
Protein kinase signaling pathways in metastatic uveal melanoma
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Purpose: Uveal Melanoma (UM) is the most common intra-ocular tumor in adults. Mutations in the Gαq family members GNAQ and GNA11 are present in 80% of UM tumors whereas the BRAF mutations are less common (15%). Consequently, Protein Kinase signaling pathways are dysregulated in UM cells and may trigger cell proliferation, survival, invasion and metastasis. Furthermore, exosomes –small nanoparticles containing RNA and proteins-, may be released by the primary tumor in order to prepare the metastatic niche in the liver before the arrival of metastatic cells. We aim to characterize the dysregulated Protein Kinase signaling pathways associated to the metastatic UM cells to identify specific targets to treat these patients.
Methods: To this aim, we used a panel of 7 UM cell lines harboring either GNAQ /GNA11 or BRAF mutations to analyse the exosome production in culture and their "resistance to anoikis", a hallmark of circulating tumor cells. We performed an *in vitro* screening using a kinase inhibitor library (Screen-Well® Kinase Inhibitor Library; Enzo, Catalog # BML-2832-0100) containing inhibitors that either

might block kinase activation or proteins that are upstream from their kinase target. Cell proliferation after incubation for 48h in the presence of inhibitors (10μM) was assessed by WST-1 metabolic assay and cell viability. Changes in cell morphology were detected under microscopy after staining with cristal violet. Cell cycle was analysed by flow cytometry. We confirmed the results obtained from the screening by Western Blot.
Results: The cell line SP6.5 produced the lowest amount of exosomes/10^6cells followed by UMA and OMM-1. In contrast, Mel270 cells and the metastatic cell lines OMM-2.3 and 2.5 resulted as the major producers of exosomes.
We found a consistent activation of Protein Kinase C (PKC), MAPK and FAK/Src in both GNAQ /GNA11 and BRAF mutant genotypes. Specific inhibitors of such pathways suppressed the corresponding signaling. Importantly, SB-203580 (MAPK inhibitor) was not effective in anoikis resistant Mel 270 $GNAQ^m$ cells but significantly affected the sensitive cells. An hyperactivation of the Akt/PI3K was observed in these cells but not in UMA $BRAF^m$ cells. BAY-117082 (inhibitor of NF-*k*B activation) significantly decreased the survival of UMA cells resistant to the anoikis but not the sensitive ones.
Conclusions: Our study identifies activated signaling pathways and therapeutic inhibitors for metastatic UM cells.
Commercial Relationships: Jose M. M. Caminal, None; **Eduard Cabre**, None; **Antonia Vinyals**, None; **Montse Goma**, None; **Josep M Piulats**, None; **Mar Varela**, None; **Daniel Lorenzo**, None; **Olaia Subira**, None; **Maria Jose Paules**, None; **Lluis Arias**, None; **Marc Rubio**, None; **Estefania Cobos**, None; **Pere Garcia-Bru**, None; **Bruno Medeiros**, None; **Noel Padron**, None; **Angels Fabra**, None
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Presentation Time: 8:30 AM–10:15 AM
Outcomes of quercetin treatment on the metastatic profile of a uveal melanoma cell line
Laurenz L. Sonnentag, Anna Kraus, Mahdy Ranjbar, Julia Lueke, Salvatore Grisanti, Aysegul Tura. Department of Ophthalmology, University of Luebeck, Luebeck, Germany.
Purpose: To evaluate the efficacy of the dietary flavonoid quercetin on suppressing the viability, proliferation, and migration of the uveal melanoma cells as well as the expression of major proteins involved in metastatic activity.
Methods: Cultures of 92.1 cells were incubated with quercetin at a concentration range of 1-100 μM. Viability was analysed by the MTT-test, Live-Dead staining, and TUNEL-assay. Cell proliferation was assessed by BrdU incorporation and Ki-67 immunostaining. Migration was analyzed by the wound healing assay. Protein expression was analysed by immunocytochemistry and -blotting.
Results: Quercetin resulted in a dose-dependent reduction in cell proliferation, survival, and migration, with the effects becoming significant ($P<0.05$) from 25 μM onwards. Quercetin also interfered with the expression of the insulin-like growth factor-1 receptor on the cell surface, possibly by preventing the upregulation of the transcriptional coactivator YAP-1 in the nucleus.
Conclusions: These findings demonstrate the efficacy of quercetin as a novel therapy alternative for suppressing the metastatic potential of uveal melanoma cells.
Commercial Relationships: Laurenz L. Sonnentag, None; **Anna Kraus**, None; **Mahdy Ranjbar**, None; **Julia Lueke**, None; **Salvatore Grisanti**, None; **Aysegul Tura**, None

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Presentation Time: 8:30 AM–10:15 AM
Expression of the programmed cell death ligand 1 (PD-L1) on uveal melanoma cells with Monosomy-3
Aysegul Tura, Anna Kraus, Mahdy Ranjbar, Julia Lueke, Salvatore Grisanti. Department of Ophthalmology, University of Luebeck, Luebeck, Germany.
Purpose: The programmed cell death ligand 1 (PD-L1) is a glycoprotein, which is upregulated on most tumor cells. PD-L1 can promote immune escape via binding to the PD-1 receptor on lymphocytes and rendering the T cells unresponsive to tumor cells. The expression of PD-L1 can be induced by various proinflammatory molecules, with interferon(IFN)-gamma being the most potent inducer. In this study, we analyzed the extent of IFN-gamma dependent PD-L1 upregulation in uveal melanoma (UM) cells with regard to the presence of monosomy-3. We also examined the efficacy of the dietary flavonoid quercetin in suppressing the PD-L1 upregulation.
Methods: UM cells were isolated from the primary tumor of a patient (female, 39 years) operated in our clinic, who developed liver metastases within 2 years after enucleation. Cultured cells (Passages 1-3) were dissociated by dispase, seeded on to 8-well chamber slides, and incubated in culture medium (RPMI-1640 supplemented with 10% fetal bovine serum, 2 mM L-Glutamine and penicillin/ streptomycin) with or without IFN-gamma (25 ng/ml) and Quercetin (100 μM) for 2 days. An Immuno-FISH assay was developed for the simultaneous detection of the PD-L1 protein and chromosome-3.
Results: Monosomy-3 was detected in a minimum of 60% of the cultured cells. Control cells incubated in normal culture medium exhibited a weak PD-L1 expression on the cell surface. IFN-gamma led to a significant but uneven upregulation in PD-L1 expression, with some cells exhibiting a particularly high level of this protein. Immuno-FISH assays demonstrated a higher degree of IFN-gamma-dependent PD-L1 upregulation on most cells with Monosomy-3 compared to the cells with Disomy-3. Quercetin could significantly suppress the IFN-gamma dependent PD-L1 upregulation on UM cells.
Conclusions: The higher level of PD-L1 upregulation in the UM cells with Monosomy-3 might be one of the mechanisms underlying the elevated metastatic potential of these cells. Treatment with the dietary flavonoid quercetin can be considered as a novel therapy option to prevent the escape of these cells from the immune system.
Commercial Relationships: Aysegul Tura, None; **Anna Kraus**, None; **Mahdy Ranjbar**, None; **Julia Lueke**, None; **Salvatore Grisanti**, None

Program Number: 3968 **Poster Board Number:** A0255
Presentation Time: 8:30 AM–10:15 AM
Uncovering the Mechanisms Behind Hepatic Microenvironment Remodeling in Metastatic Uveal Melanoma
Ioana Fugaru[1, 2], Julie Bérubé[2], Narjes Babchia[3], Cédric Coulouarn[3], Frédéric Mouriaux[3], Solange Landreville[1, 2]. [1]Université Laval, Quebec, QC, Canada; [2]Centre de Recherche du CHU de Québec-Université Laval, Quebec, QC, Canada; [3]INSERM UMR991, Université de Rennes 1, Rennes, France.
Purpose: 50% of uveal melanoma (UM) patients develop liver metastases by 10 years after the diagnosis of the primary tumor. Powerful prognostic tools help determine the likelihood of metastatic progression, however, no cure is currently available for patients with metastatic disease. Activated hepatic stellate cells (HSCs) are involved in hepatic fibrosis during the metastatic progression of colorectal and pancreatic cancers; yet their role in the metastatic progression of UM remains elusive. We postulate that HSCs

enable metastatic UM cells (UMCs) to exit dormancy and enter a proliferative state by secreting cytokines and growth factors and by remodeling the hepatic extracellular matrix (ECM). Our research project aims to develop both 2D and 3D models to investigate ECM remodeling processes and bilateral interactions between HSCs and UMCs.
Methods: Matrix sheets were synthetized by HSCs using the self-assembly approach of tissue engineering. UMCs were embedded into the 3D hepatic stromas and matrix proteins were analyzed by confocal microscopy. In addition, migration tests, cytokine proteome profiling, and gene profiling were performed in order to study the bilateral interactions between HSCs and primary or metastatic UMCs.
Results: We demonstrated that UMCs altered the ECM architecture of reconstructed HSC stromas. We observed a significant increase of transcripts/proteins associated to matrix remodeling such as fibronectin, lysyl oxidase like 4, chitinase 3 like 1 and plasminogen activator urokinase receptor in samples derived from HSCs co-cultured with metastatic UMCs. In HSCs/UMCs co-cultures, cell migration was faster and the secretome showed an increase of pro-tumoral cytokines, such as VEGF and GDF-15.
Conclusions: Co-culturing HSCs with metastatic UMCs resulted in ECM remodeling and increased secretion of pro-inflammatory, pro-angiogenic and pro-invasive cytokines. Our project aims to identify molecules involved in the development of a permissive metastatic microenvironment in the liver. By targeting components of the stromal response of HSCs, we might maintain liver micrometastases in permanent dormancy and have a major impact on patient survival.
Commercial Relationships: Ioana Fugaru; **Julie Bérubé**, None; **Narjes Babchia**, None; **Cédric Coulouarn**, None; **Frédéric Mouriaux**, None; **Solange Landreville**, None
Support: Fonds de recherche du Québec – Santé (FRQS), Vision Health Research Network, Canada Foundation for Innovation

Program Number: 3969 **Poster Board Number:** A0256
Presentation Time: 8:30 AM–10:15 AM
Liver Metastasis in Uveal Melanoma: Trends in Detection and Treatment
Lucy Xu, Arun D. Singh. Cole Eye Institute, Cleveland, OH.
Purpose: The liver is the most common site of uveal melanoma metastasis. There is a lack of consensus on screening and treatment guidelines for liver metastasis. We report the trends in uveal melanoma liver metastasis detection, treatment and outcomes.
Methods: This was a retrospective observational study of patients with uveal melanoma metastatic to the liver from 2003-2016. Data collection included type of uveal melanoma, time to liver metastasis, detection method, treatment of liver metastasis, and time to death. Trends in detection and treatment of liver metastasis were divided between time periods 2003-2006, 2007-2010 and 2011-2016. Continuous variables were compared with Student's t-test.
Results: 73 patients were included in the study. Choroidal melanoma was the most common type of uveal melanoma (n=37, 50%). The average time from uveal melanoma diagnosis to liver metastasis was 32±39 (standard deviation) months. Most of the cases of metastatic disease were diagnosed with routine surveillance using liver ultrasound (n=49, 67%). Initial treatment was chemotherapy for 15 patients (21%) and 15 received radioembolization. 11 patients (15%) chose palliation, 11 received radiofrequency ablation, 5 received immunotherapy, 2 received liver resection, 7 had combination therapy and 7 patients did not follow up within our system. Routine surveillance with ultrasound was the most frequent mode of detection across the three time periods (2003-2006, 2007-2010 and 2011-2016). Systemic chemotherapy was the most common treatment for 2003-2010. Between 2011-2016, radioembolization (with and without immunotherapy) was the most common treatment. Most patients in the study had died (n=56, 77%) with the average time from liver metastasis diagnosis to death being 15±13 months. There was a statistical difference between the treated group (n=55) and palliative group (n=11) in the survival duration (17.6 months vs 4.6 months) following diagnosis of liver metastasis ($p<0.01$).
Conclusions: Routine imaging surveillance with ultrasound is the most common method of liver metastasis detection. Systemic chemotherapy was the most common form of treatment for liver metastasis but recently has been surpassed by radioembolization. Treatment of liver metastasis is associated with longer survival times. Future studies with larger patient populations are needed to elucidate detection and treatment guidelines for liver metastasis of uveal melanoma.
Commercial Relationships: Lucy Xu, None; **Arun D. Singh**, None
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Program Number: 3970 **Poster Board Number:** A0257
Presentation Time: 8:30 AM–10:15 AM
Local recurrence of uveal melanoma increases the risk of metastatic disease in patients with abnormal copy number of chromosome 8
Mette Bagger[1, 2], Charlotte A. Espensen[1], Morten T. Andersen[2], Mette K. Andersen[2], Steffen Heegaard[1], Jens F. Kiilgaard[1].
[1]Opthalmology, Copenhagen University Hospital, Gentofte, Denmark; [2]Clinical Genetics, Copenhagen University Hospital, Copenhagen, Denmark.
Purpose: Local recurrence (LR) has recently been shown to be an important risk factor for metastatic disease in uveal melanoma (UM) independent of tumor size. However, the relationship between adverse chromosomal aberrations and LR is not known. We performed a single center retrospective consecutive cohort study to evaluate if the risk of LR and the risk of metastatic UM in the presence of LR were affected by genetic status of chromosome 8.
Methods: A consecutive cohort of 216 UM patients who underwent eye conserving treatment at Copenhagen University Hospital from 2008 through 2015 was included in the study and followed up until either death or December 2016. Copy number (CN) of chromosome 8 as assessed by Fluorescence in situ hybridization (FISH) and/or Multiplex ligation-dependent probe amplification (MLPA) and secondary treatment (enucleation or brachytherapy) due to LR were registered. The association between LR and CN of chromosome 8 was evaluated with the X^2 test. The risk of metastatic disease in relation to LR and genetic status of chromosome 8 was evaluated using Kaplan Meier survival analysis and the log rank test.
Results: Median follow up time was 4.1 years (range 0.07- 8.9, interquartile range: 2.4-6.3). LR was observed in 32 patients (15.0 %) with a median time from primary treatment to LR of 19.8 months (range 3.9- 91.0; interquartile range: 9.4-32.7). Genetic status of chromosome 8 was available in 23 of the LR cases and was normal in 12 cases and abnormal in 11 cases ($p=0.93$). There was no increased risk of LR in the presence of abnormal CN of chromosome 8 (relative risk=1.006 (95% CI 0.88-1.15)). We found a tendency ($p=0.11$) towards decreased survival in patients with LR compared to patients with no LR (UM specific 5-year survival rate of 75.1% (95%CI: 0.59-0.91) vs.85.6% (95% CI: 0.80-0.91) respectively). No patients with LR and normal CN of chromosome 8 developed metastatic disease in the study period.
Conclusions: An association between abnormal CN of chromosome 8 and LR was not found. LR in UM patients seems to increase

the risk of metastatic disease in patients with abnormal CN of chromosome 8 while we observed no decreased survival in patients with LR and normal CN of chromosome 8.
Commercial Relationships: Mette Bagger, None; **Charlotte A. Espensen**, None; **Morten T. Andersen**, None; **Mette K. Andersen**, None; **Steffen Heegaard**, None; **Jens F. Kiilgaard**
Support: Fight for Sight, Denmark, The research grant of Rigshospitalet

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Presentation Time: 8:30 AM–10:15 AM
Hepatic arterial melphalan perfusion of liver predominant uveal melanoma metastasis
Annahita Amireskandari[1], Georgia Beasley[2], Anne Kunkler[1], Srinivas Kondapalli[1], Thomas Olencki[3], Mark Bloomston[4], Carl Schmidt[2], Mohamed H. Abdel-Rahman[1], Robert Pilarski[5], Frederick Davidorf[1], Colleen M. Cebulla[1]. [1]Ophthalmology, Ohio State University, Columbus, OH; [2]Surgical Oncology, Ohio State University, Columbus, OH; [3]Medical Oncology, Ohio State University, Columbus, OH; [4]21st Century Oncology, Fort Myers, FL; [5]Division of Human Genetics, Ohio State University, Columbus, OH.
Purpose: To evaluate the benefits and complications of patients undergoing isolated hepatic perfusion (IHP) for metastatic uveal melanoma (UM) at a single center
Methods: Retrospective chart review of patients known to the senior author with liver predominant UM metastasis that received open isolated hepatic arterial melphalan perfusion from 2011 to 2015.
Results: 6 patients were evaluated; all had primary ciliary body or choroidal melanoma (AJCC T1b-T4b). 4 were treated with enucleation and 2 with brachytherapy. The median time from diagnosis of uveal melanoma to liver metastases was 22.0 months (2.5-78.1). The median age at diagnosis with liver metastasis was 61 years (50-72). 5 of the 6 patients underwent IHP while 1 had an aborted procedure secondary to incompatible anatomy. Median duration of follow-up after IHP was 28.3 months (17.3-61.9). 2 of the 5 patients had complications including intraoperative splenic injury requiring splenectomy, post operative respiratory compromise, altered mental status, SIRS, acute renal insufficiency, and pancreatic fat necrosis. 3 of the 5 patients had complete response (CR), and 2 showed a partial or stable response. 1 of the 5 patients with CR had extrahepatic progression to bone after 23.9 months (and later to brain, orbit, and lungs after 26.4 months). The other 4 patients had hepatic progression (1 had hepatic and extrahepatic to lungs) after a median time of 20.0 months (13.6-57.3) from IHP. Median overall survival from time of diagnosis of liver metastases to present was 30.2 months (19.3-65.0).
Conclusions: This subset of patients treated with IHP had 30.2 month median survival. Median survival of patients with metastatic uveal melanoma noted in the literature is 2-12-months. The outcomes of the additional patients from the institution receiving IHP for metastatic UM (n=5) will be evaluated. A correlation of outcomes with the common somatic UM mutations will be performed.
Commercial Relationships: Annahita Amireskandari; **Georgia Beasley**, None; **Anne Kunkler**, None; **Srinivas Kondapalli**, None; **Thomas Olencki**, None; **Mark Bloomston**, None; **Carl Schmidt**, None; **Mohamed H. Abdel-Rahman**, None; **Robert Pilarski**, None; **Frederick Davidorf**, None; **Colleen M. Cebulla**, None
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Presentation Time: 8:30 AM–10:15 AM
DECIPHERING THE ROLE OF EXTRACELULAR VESICLES IN HUMAN UVEAL MELANOMA
Maria Santiago-Varela[1], Diego Perez-Sotelo[2], Nerea Lago-Baameiro[2], Manuel F. Bande[1], Susana Bravo[2], Francisco Ruiz-Oliva[1], María José Blanco[1], Maria Pardo[2], Antonio Piñeiro[1]. [1]Hospital de Conxo, Santiago de Compostela, Spain; [2]Fundacion Ramon Dominguez, Santiago de Compostela, Spain.
Purpose: Tumor-released extracellular vesicles (EVs) and their role on cancer pathogenesis has created great expectation as a very sophisticated way of intercellular signaling favoring tumor development and progression. Moreover, EVs may become an important source of cancer biomarkers. Since there is barely any knowledge about uveal melanoma (UM) secreted EVs, neither about their role in this ocular tumor, we aim to isolate and characterize uveal melanoma shed EVs as potential non-invasive uveal melanoma prognostic trackers.
Methods: UM-A human melanoma cell line was labeled during 72 hours by CILAIR: *Comparison of Isotope-Labeled Amino acid Incorporation Rates* with Arg and Lys (L-[13C6]-Lys/L-[13C6,15N4]-Arg). Labeled serum deprived secretomes were collected and ultracentrifugated for EVs isolation and characterization by immunoblotting, light scattering and electronic microscopy. Labeled secretomes and isolated labeled vesicles were processed for LC-MALDI protein identification followed by functional analysis.
Results: Uveal melanoma cells in culture shed exosomal extracellular vesicles of 100 nm positive for CD9 and CD63. Accordingly, cellular component classification after mass spectrometry analysis of isolated vesicles shows that 80% of identified proteins were exosomal; others were classified as from extracellular matrix or extracellular space. From the former, 50% were isotopically labeled showing that quantitative proteomics analysis by CILAIR proves to be an effective method for EVs characterization avoiding external contaminants. UM exosome proteome functional analysis reveals proteins implicated in cell growth and maintenance, protein metabolism, and angiogenesis and cell surface interactions integrins.
Conclusions: We demonstrate for the first time the secretion of vesicles of exosomal nature from UM cells. The proteomics characterization of UM exosomes suggest their implication in tumor-metastatic niche preparation and therefore reveals new potential therapeutic targets in uveal melanoma.
Commercial Relationships: Maria Santiago-Varela; **Diego Perez-Sotelo**, None; **Nerea Lago-Baameiro**, None; **Manuel F. Bande**, None; **Susana Bravo**, None; **Francisco Ruiz-Oliva**, None; **María José Blanco**, None; **Maria Pardo**, None; **Antonio Piñeiro**, None