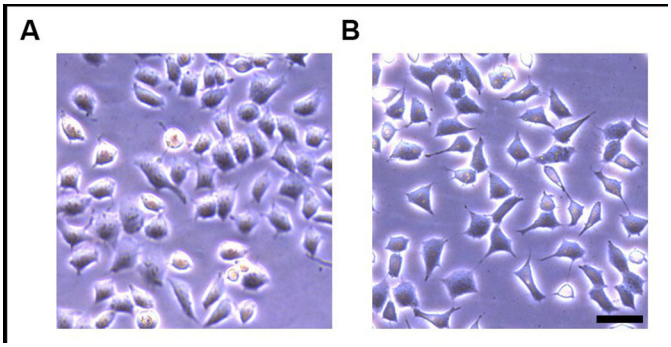
242 Retinoblastoma: Basic/Translational
Monday, May 08, 2017 11:00 AM–12:45 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 1770–1781/A0410–A0421
Organizing Section: Anatomy and Pathology/Oncology

Program Number: 1770 **Poster Board Number:** A0410
Presentation Time: 11:00 AM–12:45 PM
Pre-clinical Model of Retinoblastoma Vitreous Seeds
Andrew S. Irvine[1], Zachary K. Goldsmith[1], Rachel Brennan[1, 2], Matthew W. Wilson[1, 3], Vanessa M. Morales[1, 4]. [1]Ophthalmology, Hamilton Eye Institute, UTHSC, Memphis, TN; [2]Oncology, St. Jude Childrens Research Hospital, Memphis, TN; [3]Surgery, St. Jude Childrens Research Hospital, Memphis, TN; [4]Microbiology, Immunology and Biochemistry, UTHSC, Memphis, TN.
Purpose: The biggest challenge in treatment of retinoblastoma (Rb) is the presence of vitreous seeds. Vitreous seeding has a poorer prognosis for ocular salvage as reflected by multiple Rb classification systems. There is a lack of *in vitro* models of Rb vitreous seeds. Here, we adapt our *in vitro* 3D culture system to recreate Rb seeds in a vitreous-like microenvironment for more in-depth study.
Methods: Monocultures and co-cultures of CFSE-fluorescently labeled Y79 cells and human primary retinal microvascular endothelial cells (hRECs) were 3D cultured using magnetic levitation to re-create Rb seeds in the vitreous. We were able to visualize the growth of seeds over time *in vitro* by confocal microscopy.
Results: We generated *in vitro* Rb seeds similar to those found in Rb patients. The cultures were kept for over 2-weeks. Moreover, we were able to perform histological assessment to compare the 3D generated and *ex vivo* isolated seeds.
Conclusions: We have generated a pre-clinical model to investigate Rb seeds *in vitro* by using magnetic 3D cultures. Our novel methodology of fluorescently labeling the cells prior to magnetization allows isolation of both cell types to further characterize cells within the Rb microenvironment.
Commercial Relationships: Andrew S. Irvine, None; **Zachary K. Goldsmith**, None; **Rachel Brennan**, None; **Matthew W. Wilson**, None; **Vanessa M. Morales**, None
Support: Research to Prevent Blindness

Program Number: 1771 **Poster Board Number:** A0411
Presentation Time: 11:00 AM–12:45 PM
An Orthotopic Transplantation Model of Retinoblastoma in Beagles Mimicking Vitreous Seeds: A Novel Gateway for Investigation of Anticancer Drugs
Dong Hyun Jo[3], Jin Hyoung Kim[2, 3], Chang Sik Cho[2, 1], Young Suk Yu[2, 4], Jeong Hun Kim[2, 1]. [1]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea (the Republic of); [2]Fight against Angiogenesis-Related Blindness (FARB) Laboratory, Clinical Research Institute, Seoul National University Hospital, Seoul, Korea (the Republic of); [3]Tumor Microenvironment Research Center, Global Core Research Center, Seoul National University, Seoul, Korea (the Republic of); [4]Department of Ophthalmology, Seoul National University College of Medicine, Seoul, Korea (the Republic of).
Purpose: To develop a novel orthotopic transplantation model of retinoblastoma model in beagles which mimics vitreous seeds.
Methods: SNUOT-Rb1 and Y79 cells from 2 different retinoblastoma cell lines were injected into the vitreous cavity of beagles. At 6 weeks after the injection, the eyes were enucleated and processed for further analyses. Retinoblastoma cells isolated from the vitreous cavity were evaluated for proliferation and retinoblastoma cell markers to confirm that the retinoblastoma cells maintained their characteristics.
Results: At 6 weeks after the injection of SNUOT-Rb1 and Y79 cells, the clumps of retinoblastoma cells were evident in the vitreous cavity of beagles. Surprisingly, the isolated cells from the clumps demonstrated similar shapes to retinoblastoma cells and the viability over 98%. Further proliferation assay, western blotting, and quantitative real-time polymerase chain reaction also showed that isolated cells possessed their characteristics as retinoblastoma cells.
Conclusions: This beagle retinoblastoma model mimics vitreous seeds in retinoblastoma patients. We expect that this model will be utilized for the development of novel therapeutic options for retinoblastoma.

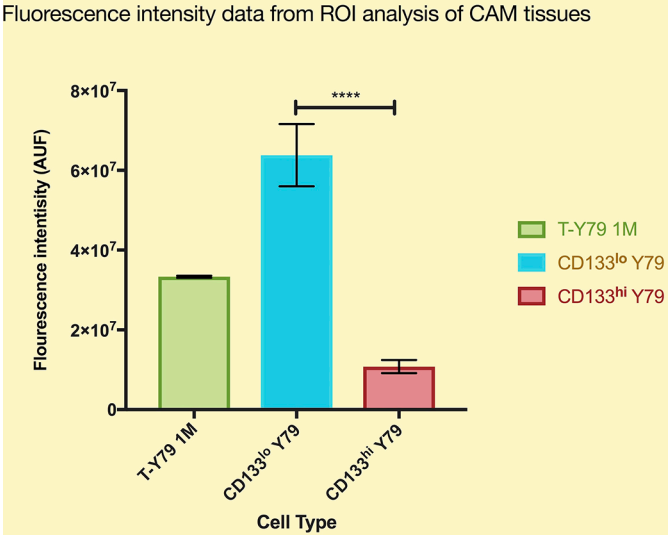


Representative photographs of SNUOT-Rb1 cells before the injection into the vitreous cavity (A) and isolated from the vitreous cavity after 6 weeks from the injection (B). Scale bar, 50 μm.
Commercial Relationships: Dong Hyun Jo, None; **Jin Hyoung Kim**, None; **Chang Sik Cho**, None; **Young Suk Yu**, None; **Jeong Hun Kim**, None

Program Number: 1772 **Poster Board Number:** A0412
Presentation Time: 11:00 AM–12:45 PM
Chick Embryo CAM model of Retinoblastoma
Geeta K. Vemuganti[1], Rohini M. Nair[1], Sucharita G[1], Narayana RVL[1], Swathi Kaliki[2, 3]. [1]School of Medical Sciences, University of Hyderabad, Hyderabad, India; [2]The Operation Eyesight Universal Institute for Eye Cancer, L V Prasad Eye Institute, Hyderabad, India; [3]Ophthalmic Pathology Laboratory, L V Prasad Eye Institute, Hyderabad, India.
Purpose: Development of animal models for Retinoblastoma (Rb) that recapitulates the tumor phenotypically and functionally has been challenging despite advances in molecular biotechnology. This study attempts to develop an *in vivo* model that is easy to establish, visualize and assess the *in vivo* behavior of RbY79 cell line and $CD133^{lo/hi}$ populations using the experimental system of Chick Embryo Chorio-allantoic Membrane model (CE-CAM).
Methods: Embryonated eggs of Gramapriya breed (G.gallus), procured after IAEC approval, were incubated at 38°C/60%RH for 8 days, after which a small window was made to introduce tumor cells. Y79 cells were evaluated for CD133 marker and sorted using MACS. The 3 groups of CM-Dil labelled cells(10^6 each): Total Y79 (GroupA, n=5), $CD133^{lo}$(GroupB, n=6) and $CD133^{hi}$(GroupC, n=5) in Matrigel, were placed on abraded CAM surface of E8 embryos, incubated and sacrificed after 1 week. The CAM was dissected and evaluated by confocal microscopy for tumor cells followed by routine histology. *In vivo* imaging of whole embryos was done to detect distant metastasis. The fluorescence images were quantified by ImageJ(AUF) and statistical analysis by GraphPad Prism.
Results: RbY79 cell line revealed 16.1±0.2% $C133^{lo}$ and 83.25±0.85% $CD133^{hi}$ cells which were sorted using MACS with

over 90%purity and 80% viability. Survival % of the embryos were 42.5±17.5, 60.5±10.5 and 75.5±4.5 respectively for Groups A,B and C. The tumors formed pinkish-white raised wet perivascular nodules with feeder vessels on the CAM. Fluorescence intensity analysis of CAM tissues showed that GroupB (AUF=$6.3*10^7±7.7*10^6$) had higher localization of cells when compared to GroupA ($p=0.1$, AUF=$3.3*10^7±0.2*10^6$) and GroupC ($p<0.0001$,A UF=$1.08*10^7±1.6*10^6$). Fluorescence signal from *in vivo* imaging of the embryos was noted predominantly in abdominal area in all groups but GroupB embryos showed intense and more spread out signal compared to Groups A and C($p=0.8;0.5$) with additional evidence of fluorescence in the cephalic region hinting at possible metastasis, as confirmed by histology.

Conclusions: *In vivo* tracking of RbY79 cell line in CE-CAM model demonstrated formation of tumor nodules in CAM with preliminary evidence of metastasis to the embryo. The study also provides proof that the $CD133^{lo}$ cells show higher propensity to form tumor deposits compared to $CD133^{hi}$ cells, suggesting they are possibly endowed with cancer stem cell like properties in Rb, which needs to be further validated.



Commercial Relationships: Geeta K. Vemuganti, None; **Rohini M. Nair**, None; **Sucharita G**, None; **Narayana RVL**, None; **Swathi Kaliki**, None
Support: UPE-II and DST-PURSE II grant, University of Hyderabad

Program Number: 1773 **Poster Board Number:** A0413
Presentation Time: 11:00 AM–12:45 PM
The human retinoblastoma WERI-Rb1 cell line as an *in vitro* model for photoreceptor-specific AAV transgene expression
Cristina Martinez-Fernandez de la Camar[1], Maria I. Patrício[1, 2], Michelle E. McClements[1], Alun R. Barnard[1, 2], Robert E. MacLaren[1, 2]. [1]Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom; [2]John Radcliffe Hospital, Oxford Eye Hospital, Oxford University Hospitals NHS Trust, Oxford, United Kingdom.
Purpose: The development of vectors encoding therapeutic genes driven by human photoreceptor-specific promoters is of special interest to increase the efficacy and the safety of gene therapy treatment in photoreceptor degenerations, such as retinitis pigmentosa. At present, however, there is no regulatory approved *in vitro* model to confirm photoreceptor-specific transgene expression with adeno-associated viral (AAV) vectors. Hence the development of a robust and reproducible *in vitro* system would facilitate progress towards clinical trials.
Methods: In this study, we used the retinoblastoma-derived WERI-Rb1 cell line to establish an *in vitro* model for the expression of a green fluorescent protein (GFP) reporter gene driven by the human rhodopsin kinase (GRK1) or chicken β-actin (CAG) promoter. Recombinant AAV2/8 vectors were prepared using iodixanol gradients (rAAV2/8.CAG.GFP and rAAV2/8.GRK1.GFP). Since several proteins activated during cellular DNA replication and transcription have been reported to interfere with viral DNA replication, we used different enzymatic inhibitors to improve AAV transduction. GFP expression levels were monitored overtime using epifluorescence microscopy.
Results: Drug toxicity was first assessed using a MTT cell viability assay to find the highest non-toxic concentration. A range of multiplicities of infection (MOI) from 2,000 to 50,000 was tested to give the best transduction efficiency, having used rAAV2/8.CAG.GFP as a positive control. Epifluorescence microscopy analysis revealed that the inhibition of some of these proteins involved in DNA metabolism such as topoisomerases increases the GFP expression under the human photoreceptor-specific promoter GRK1.
Conclusions: The human GRK1 promoter delivered by the AAV2/8 vector can be assessed in human WERI cells. This study provides a helpful human *in vitro* model to test the expression of therapeutic genes specifically in photoreceptors using the human GRK1 promoter delivered by the AAV serotype 8 vector, thus making available a reliable and specie-specific preclinical model to validate therapeutic vectors.
Commercial Relationships: Cristina Martinez-Fernandez de la Camar, None; **Maria I. Patrício**, None; **Michelle E. McClements**, None; **Alun R. Barnard**, Nightstarx Ltd (C); **Robert E. MacLaren**, Nightstarx Ltd (C)
Support: Nightstarx Ltd and the Royal College of Surgeons of Edinburgh

Program Number: 1774 **Poster Board Number:** A0414
Presentation Time: 11:00 AM–12:45 PM
A non-cytotoxic compound blocks angiogenesis and decreases tumor burden in the TAg-RB retinoblastoma mouse
Timothy W. Corson, Christian Briggs, Rania S. Sulaiman, Asmaa Mahoui, Kamakshi Sishtla, Mehdi Shadmand. Ophthalmology, Indiana University School of Medicine, Indianapolis, IN.
Purpose: Cytotoxic systemic or local chemotherapy is a mainstay of retinoblastoma therapy but is associated with significant side effects. Antiangiogenic therapy could avoid some of this toxicity. We developed the novel antiangiogenic compound SH-11037, which likely acts as a soluble epoxide hydrolase inhibitor. SH-11037 blocks angiogenesis in vitro and in murine ocular neovascularization models, making it a candidate for antiangiogenic retinoblastoma therapy. Here, we explored the effects of SH-11037 on retinoblastoma cells in culture and as a treatment for retinoblastoma in the TAg-RB transgenic mouse model.
Methods: We assessed the effects of SH-11037 on proliferation of Y79 retinoblastoma cells and ARPE-19 “normal” retinal pigment epithelial cells by alamarBlue proliferation assays. For therapeutic evaluation, TAg-RB mice were intravitreally injected with SH-11037 or vehicle to a final vitreous concentration of 10 μM weekly starting at 7 or 8 weeks of age with sacrifice and enucleation at 10 or 11 weeks. We followed tumor progression by optical coherence tomography in vivo and histopathological analysis post-mortem. Percent tumor burden in each eye was compared between cohorts and treatments by two-way ANOVA.

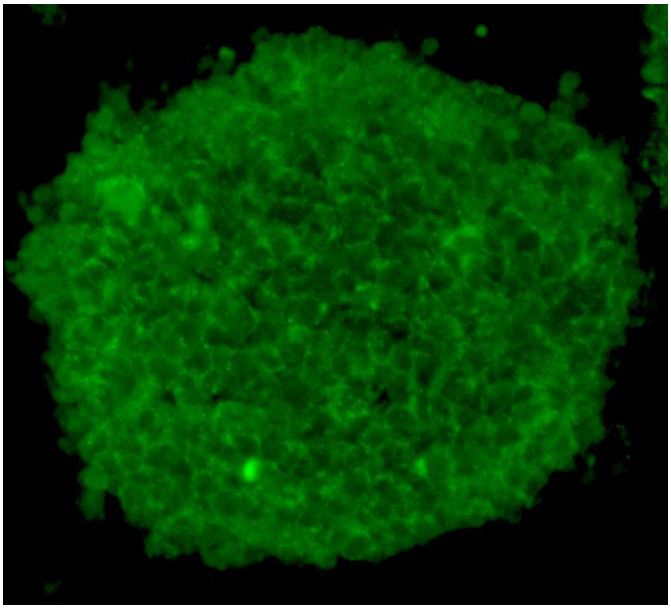
Results: SH-11037 did not block the growth of retinoblastoma or retinal pigment epithelial cells in culture, suggestive of a lack of toxicity. However, in transgenic mice with retinoblastoma, SH-11037 significantly reduced tumor burden over vehicle (up to 26% reduction, p=0.022). No adverse effects were observed with weekly compound injections.
Conclusions: SH-11037 is not toxic to tumor or healthy cells of the eye, but when administered by intravitreal injection may be a candidate for retinoblastoma treatment by blocking angiogenesis. Further validation of the possibility of intravitreal, antiangiogenic retinoblastoma therapy using other drugs and other preclinical models is indicated.
Commercial Relationships: Timothy W. Corson, US 2016/0060241 A1; PCT/US2016/062851 (P); **Christian Briggs**, None; **Rania S. Sulaiman**, PCT/US2016/062851 (P); **Asmaa Mahoui**, None; **Kamakshi Sishtla**, None; **Mehdi Shadmand**, None
Support: St. Baldrick's Foundation, NIH R01EY025641, Research to Prevent Blindness, Inc.

Program Number: 1775 **Poster Board Number:** A0415
Presentation Time: 11:00 AM–12:45 PM
Effect of the pre-treatment with the radioprotector ortho-phospho-L-tyrosine (pTyr) in a xenograft retinoblastoma mouse model
Alexander V. Tschulakow[1], H. Peter Rodemann[2], Stephan M. Huber[3], Monika Rittgarn[1], Dominik Klumpp[3], Benjamin Steegen[3], Ulrich Schraermeyer[1], Sylvie Julien-Schraermeyer[1]. [1]Division of Experimental Vitreoretinal Surgery, Center of Ophthalmology, Tuebingen, Germany; [2]Division of Radiobiology & Molecular Environmental Research, Department of Radiation Oncology, University Hospital, Tuebingen, Germany; [3]Department of Radiation Oncology, University Hospital, Tuebingen, Germany.
Purpose: Retinoblastoma (Rb) is the most frequent intraocular tumor in children and if left untreated, can cause death. Like most head and neck tumors, Rb is sensitive to radiotherapy (RT). However, the residual risk of a recurrence and development of treatment-induced secondary tumors still remains. In a previous study (Tschulakow et al., IOVS 2015; 56(7):72), we analyzed the ability of the radioprotector ortho-phospho-L-tyrosine (pTyr) to prevent radiation therapy-induced secondary tumors in Rb+/- mice, a model for Rb patients with hereditary Rb who are predisposed to secondary malignancies after RT. We showed that the pre-treatment with pTyr significantly reduces the negative effects of radiation on the hair coat as well as in the retina (retinal thickness reduction and photoreceptor loss) of Rb+/- mice. However, independent of the pre-treatment with pTyr, no secondary tumors were detectable.
In this study, we used a new xenograft Rb mouse model, which was developed by our group (Tschulakow et al., 2016), to investigate the tumor's recurrence and the occurrence of secondary tumors after radiotherapy and possible ways of preventing them by the application of pTyr.
Methods: Immune-deficient nude mice carrying orthotopic human Y79 xenograft tumors were treated by RT (15 fractions of 5 Gy 6 MV photons during 3 weeks) with and without pre-treatment with pTyr. The animals were investigated in vivo using SLO/OCT and histologically 1, 3, 6 and 9 months after RT. Success of the RT as well as radiation-induced tumor development and normal tissue radiation toxicity were evaluated as a function of pTyr treatment.
Results: In the xenografted Rb model, tumor control was reached in 16 out of 22 control eyes after RT whereas it was not observed in all of the 24 pTyr-treated eyes. No secondary tumors were detected.
Conclusions: Although pTyr has a protective effect on normal tissues, unfortunately it dramatically lowers the efficacy of RT and is therefore not suitable for RT in Rb patients.
Commercial Relationships: Alexander V. Tschulakow, None; **H. Peter Rodemann**, None; **Stephan M. Huber**, None; **Monika Rittgarn**, None; **Dominik Klumpp**, None; **Benjamin Steegen**, None; **Ulrich Schraermeyer**, None; **Sylvie Julien-Schraermeyer**
Support: Deutsche Kinder Krebs Stiftung (DKKS 2012.08).

Program Number: 1776 **Poster Board Number:** A0416
Presentation Time: 11:00 AM–12:45 PM
HER2 expression in retinoblastoma: a potential therapeutic target?
David C. Sousa[1, 2], Pablo Zoroquiain[3], Evangelina Esposito[3], Maria E. Orellana[4], Bruna Duarte Moron de Andrade[3], Miguel N. Burnier[3]. [1]Ophthalmology, Hospital de Santa Maria, Lisboa, Portugal; [2]Centro de Estudos Ciências da Visão, Universidade de Lisboa, Lisboa, Portugal; [3]McGill University Ocular Pathology Laboratory, MUHC, Montreal, QC, Canada; [4]Instituto Anatomopatológico 'Dr. José A. O'Daly', Universidad Central de Venezuela, Caracas, Venezuela, Bolivarian Republic of.
Purpose: Retinoblastoma (RB) is the most common primary intraocular malignancy in children. Current therapies are associated with both high short- and long-term morbidity. Human epidermal growth factor receptor 2 (HER2) is a transmembrane protein detected in 15%–30% of breast cancers, which has also been described in other malignancies. Recently, it was reported that a truncated version of this protein is expressed in RB, which is responsive to targeted therapies in vitro. Herein, we scored HER2 expression in RB samples and discuss its potential clinical utility.
Methods: A total of 60 RB cases and the Y79 RB cell line were evaluated for HER2 expression using a monoclonal antibody and the fully automated Ventana immunostaining system. HER2 expression was evaluated using the traditional 0 to 3+ scoring method according to the membranous staining pattern in archival formalin-fixed, paraffin-embedded RBs. A cell block was generated with the Y79 cell line, and it was scored in a similar fashion.
Results: The mean age at enucleation was 31.6 ± 31.5 months, while the mean time from diagnosis to enucleation was 11.8 ± 11.2 months (range 1–44 months). Five cases (8%) were multifocal. HER2 expression was negative in all RB cases (49 cases scored 0 and 11 scored 1+) and in the Y79 cell line.
Conclusions: Despite the large sample size, and the inclusion of a RB cell line, in our study, there was no significant expression of HER2 in RB. Based on our results, it is unlikely that anti-HER2 therapies will be effective for treating RB via the traditional pathways.
Commercial Relationships: David C. Sousa, None; **Pablo Zoroquiain**, None; **Evangelina Esposito**, None; **Maria E. Orellana**, None; **Bruna Duarte Moron de Andrade**, None; **Miguel N. Burnier**, None

Program Number: 1777 **Poster Board Number:** A0417
Presentation Time: 11:00 AM–12:45 PM
HER2 immunoreactivity and drug targeting in human retinoblastoma
Gail M. Seigel[1, 2], Pia Mendoza[3], Eszter Szalai[3], Dhaval K. Shah[4, 2], Hans E. Grossniklaus[3]. [1]Center for Hearing and Deafness, University at Buffalo, Buffalo, NY; [2]SUNY Eye Institute, Buffalo, NY; [3]Ophthalmology, Emory University, Atlanta, GA; [4]Pharmaceutical Sciences, University at Buffalo, Buffalo, NY.

Purpose: Retinoblastoma (RB) is an ocular malignancy of early childhood. Although there has been success in treating RB through enucleation, recent emphasis has been on vision-preserving chemotherapy treatments that spare the eye and preserve visual function, but may not kill chemoresistant cells. There is a need for targeted RB therapies that can address chemoresistance and provide a wider therapeutic index. One potential therapeutic target for RB is HER2, (ERBB2), a member of the epidermal growth factor family that is expressed in RB cell lines and tumors, as we have shown previously. In this study, we conducted a larger scale examination of 28 human RB tumors to address the following hypotheses: 1) HER2 is preferentially expressed in high grade, invasive tumors and 2) HER2 is sufficiently expressed in RB tumors to warrant further development of HER2-targeted RB therapies.
Methods: Twenty-eight paraffin sections of RB tumors (Groups B-D; TNM Stages pT1-pT4) were examined by HER2 immunohistochemistry, along with isotype negative controls. Fluorescence intensities in specific regions of the tumors (eg. central tumor, leading edge, vitreous seeds) were quantitated by ImageJ analysis and analyzed by Prism Software for statistical significance. Fluorescence results and clinical parameters (Group, TNM status) were correlated for each tumor.
Results: Fourteen of the 28 human RB samples show HER2 staining intensity at least two standard deviations above the mean of the negative control. Specific tumor regions were statistically more fluorescent than the negative control (p values ranging from 0.0001 to 0.042). Of the eight RB tumors with the brightest regions of fluorescence, five were staged pT3b and seven were in Group D or E, considered high grade tumors in our cohort. Only one of these tumors was not (yet) invasive. Half of the pT3b grade were HER2-high (5/10), compared to the combined lower grades of pT1-pT2a (2/10).
Conclusions: Our results suggest that HER2 is expressed at significant levels in at least half of our RB cohort, with higher levels of immunoreactivity in higher grade tumors. This information will be important for personalized medicine decisions as we develop HER2-targeted therapies for RB. In turn, this may lead to innovative and targeted drug treatment options designed to spare the eye, preserve vision and improve quality of life for RB patients.



HER2 immunoreactivity in RB

Commercial Relationships: Gail M. Seigel, None; **Pia Mendoza**, None; **Eszter Szalai**, None; **Dhaval K. Shah**, None; **Hans E. Grossniklaus**, None
Support: DKS was supported by start-up funds from the School of Pharmacy and Pharmaceutical Science at SUNY Buffalo

Program Number: 1778 **Poster Board Number:** A0418
Presentation Time: 11:00 AM–12:45 PM
The PDGF-PDGFR Signaling Pathway Regulates AKT Cell Survival and NF-kB anti-apoptotic effects in Retinoblastoma
Matthew W. Wilson[1, 2], Zachary K. Goldsmith[1], William Coppess[1], Kelley Yuan[1], Madison Ritter[1], Andrew Irvine[1], Rachel Brennan[3], Vanessa M. Morales[1, 4]. [1]Ophthal/Hamilton Eye Int, Univ of Tennessee Health Sci Ctr, Memphis, TN; [2]Surgery and Pathology, St Jude Children's Research Hospital, Memphis, TN; [3]Oncology, St Jude Children's Research Hospital, Memphis, TN; [4]Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, TN.
Purpose: Purpose: Vitreous seeding remains the greatest challenge in treatment of retinoblastoma (Rb). In part due to poor understanding of the interactions between Rb and vitreous microenvironment. Previously, we identified the PDGF-PDGFR signaling as a signaling pathway that sustains angiogenesis in both an autocrine and paracrine fashion. Here, we investigated how the PDGF-PDGFR signaling pathway regulates cell proliferation and cell survival in Rb.
Methods: Methods: We performed *in vitro* cell culture assays of Y79 Rb cells, considered the metastatic model of the disease. Cells were cultured in different conditions, which include stimulatory (recombinant human PDGF) and inhibitory (imatinib mesylate) conditions of the PDGF-PDGFR signaling pathway. Assessment of cell survival included measurement of members of the AKT signaling pathway and protein levels of the anti-apoptotic molecule BCL-2 by Western blot analyses. Nuclear translocation of the p65 subunit of NF-kB was measured by imaging flow cytometry using FlowSight®.
Results: Results: Our results suggest that PDGF-PDGFR signaling regulates Rb cell survival through AKT. Additionally, we found a significant reduction in BCL-2, concomitant with an increase in caspase-3 activity. We also found a significant decrease in the nuclear translocation of the p65 subunit of NF-kB
Conclusions: Conclusions: The outcomes of this study reveal that PDGF-PDGFR signaling regulates cell survival in addition to the previously described sustainment of angiogenesis. We found PDGF-PDGFR signaling is involved in the activation of the AKT survival pathway. Inhibition of PDGF-PDFGR signaling decreases NF-kB nuclear translocation, ultimately resulting in increased susceptibility to apoptosis.
Commercial Relationships: Matthew W. Wilson, None; **Zachary K. Goldsmith**, None; **William Coppess**, None; **Kelley Yuan**, None; **Madison Ritter**, None; **Andrew Irvine**, None; **Rachel Brennan**, None; **Vanessa M. Morales**, None
Support: Research to Prevent Blindness, Inc and St Jude Children's Research Hospital Endowed Chair in Pediatric Ophthalmology

Program Number: 1779 **Poster Board Number:** A0419
Presentation Time: 11:00 AM–12:45 PM
Inhibition of PDGFR-β Decreases Pro-Survival Signaling of Retinoblastoma *in vitro*
Madison Ritter[1], William Coppess[1], Zachary K. Goldsmith[1], Rachel Brennan[2, 1], Matthew W. Wilson[1, 3], Vanessa M. Morales[1, 4]. [1]Ophthalmology, Hamilton Eye Institute, UTHSC, Memphis, TN; [2]Oncology, St. Jude Childrens Research Hospital, Memphis, TN; [3]Surgery, St. Jude Childrens Research Hospital, Memphis, TN; [4]Microbiology, Immunology and Biochemistry, UTHSC, Memphis, TN.
Purpose: Retinoblastoma (Rb) is the most common primary intraocular malignancy in children. Although highly treatable in the United States, there is a high mortality rate in developing countries due to metastatic disease. We have previously shown that signaling through platelet-derived growth factor receptor-beta (PDGFR-β) promotes invasiveness and survival of Rb and, when PDGFR-β is inhibited, there is a decrease in proliferation.
Methods: The well-characterized Y79 Rb cell line was used and represents the metastatic form of the disease. We investigated the role of the PDGFR-β in the control of cell survival by culturing cells in the presence of imatinib mesylate (IM), an inhibitor of kinases encoded by the *BCR-ABL* and *PDGFR* oncogenes. To address the effect of IM in cell death and survival, we measured the expression of both pro- and anti-apoptotic proteins, including AKT, BCL-2, and Caspase-3 by Western blot.
Results: We found a significant reduction in BCL-2 and AKT in Rb cells treated with IM. Moreover, we found an increase in Caspase-3 in IM-treated cells.
Conclusions: Our results are consistent with our hypothesis that inhibition of PDGFR-β impacts cell survival by reduction of the anti-apoptotic protein BCL-2 and the pro-survival molecule AKT while increasing Caspase-3.
Commercial Relationships: Madison Ritter, None; **William Coppess**, None; **Zachary K. Goldsmith**, None; **Rachel Brennan**, None; **Matthew W. Wilson**, None; **Vanessa M. Morales**, None
Support: Research to Prevent Blindness

Program Number: 1780 **Poster Board Number:** A0420
Presentation Time: 11:00 AM–12:45 PM
The impact of disruption of PDGF-PDGFR-β signaling on VEGF-VEGFR signaling in retinoblastoma
William Coppess[1], Zachary K. Goldsmith[1], Madison Ritter[1], Kelley Yuan[1], Andrew S. Irvine[1], Rachel Brennan[1, 2], Matthew W. Wilson[1, 3], Vanessa M. Morales[1, 4]. [1]Ophthalmology, University of Tennessee Health Science Center, Memphis, TN; [2]Oncology, St. Jude Children's Research Hospital, Memphis, TN; [3]Surgery, St. Jude Children's Research Hospital, Memphis, TN; [4]Microbiology, Immunology, and Biochemistry, University of Tennessee Health Science Center, Memphis, TN.
Purpose: Retinoblastoma (Rb) is a common intraocular malignancy in children. Targeting of tumor vasculature is important to disrupt the growth and nourishment of tumors. Vascular endothelial growth factor (VEGF) is considered a major regulator in retinal angiogenesis. However, recent work suggests VEGF is important in neural development. Previously, we showed disruption of PDGFR-β signaling reduces Rb proliferation and invasion. Here, we investigate if there is a compensatory mechanism by VEGFR2 in the absence of PDGFR-β signaling.
Methods: We used Y79, the metastatic model of Rb, to investigate the expression and upregulation of VEGF-VEGFR2 signaling after disruption of the PDGFR-β downstream signaling. We measured VEGFA, VEGFR2 mRNA by qPCR analyses and protein levels by Western blot. We also measured the expression of these molecules by qPCR using samples from an orthotopic xenograft system.
Results: qPCR analyses showed upregulation of the VEGFR2 and VEGFA in Rb cell cultures and in the ocular tissue from an orthotopic xenograft system. When the PDGF-PDGFR-β downstream signaling was disrupted, there was no change in the VEGFR2 signaling, measured by the ratio of phosphorylated to total VEGFR2.
Conclusions: Our results showed no changes in VEGFR signaling in Y79 Rb cells after disruption of the PDGF-PDGFR-β signaling pathway. These results suggest there is no compensatory mechanism by VEGFR2 in the absence of signaling through PDGF-PDGFR-β. Modulation of PDGFR-β affects angiogenesis, but does not affect VEGF-VEGFR, which may play a role in neural development.
Commercial Relationships: William Coppess, None; **Zachary K. Goldsmith**, None; **Madison Ritter**, None; **Kelley Yuan**, None; **Andrew S. Irvine**, None; **Rachel Brennan**, None; **Matthew W. Wilson**, None; **Vanessa M. Morales**, None

Program Number: 1781 **Poster Board Number:** A0421
Presentation Time: 11:00 AM–12:45 PM
The effects of inhibition of PDGFR-β in retinal endothelial cells within the retinoblastoma tumor microenvironment
Zachary K. Goldsmith[1], William Coppess[1], Kelley Yuan[1], Andrew S. Irvine[1], Rachel Brennan[1, 2], Matthew W. Wilson[1, 3], Vanessa M. Morales[1, 4]. [1]Ophthalmology, University of Tennessee Health Science Center, Germantown, TN; [2]Oncology, St. Jude Children's Research Hospital, Memphis, TN; [3]Surgery, St. Jude Children's Research Hospital, Memphis, TN; [4]Microbiology, Immunology, and Biochemistry, University of Tennessee Health Science Center, Memphis, TN.
Purpose: Retinoblastoma (Rb) is a highly angiogenic tumor, for which anti-VEGF therapies have shown limited success in clinical setting. This illustrates the need for more novel therapeutics. Previous work from our lab showed inhibition of PDGFR-β inhibited Rb cell proliferation in vitro. However, novel therapies must not only target tumor cells but also how they affect the tumor microenvironment.
Methods: We investigated the role of the PDGFR-β in the tumor microenvironment and how inhibition of this signaling pathway impacts angiogenesis in human retinal microvascular endothelial cells (hRECs). Rb monocultures and co-cultures with hRECs were evaluated for the production of the angiogenic proteins PDGF and VEGF after treatment with imatinib mesylate, a small molecule kinase inhibitor. We performed tube formation assay in hRECs to investigate if PDGF played a role in structural integrity of the vasculature.
Results: We found that inhibition of the PDGFR-β signaling pathway through imatinib mesylate affects both paracrine and autocrine angiogenic mechanisms in Rb cells. Production of VEGF is also affected by inhibition of this signaling pathway.
Conclusions: More studies are underway to elucidate if the use of imatinib mesylate will also regulate hRECs cell survival. This is of critical importance as success of treatment also depends on the ability of the normal tissues to remain healthy after sensitization and/or killing of the Rb tumor.
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