215 Novel Imaging and Biomarkers for Ocular Tumors and Disease
Monday, May 08, 2017 8:30 AM–10:15 AM
Room 321  Paper Session
Program #/Board # Range: 1251–1256
Organizing Section: Anatomy and Pathology/Oncology

Program Number: 1251
Presentation Time: 8:30 AM–8:45 AM
Precision medicine for vitreoretinal lymphomas: new routes to targeted therapies from liquid biopsies
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Purpose: The purpose of this study is to elucidate the actionable cancer genome of vitreoretinal lymphoma (VRL).

Methods: In this retrospective study, we collected diluted vitreous biopsies from 4 patients with a high suspicion for VRL. Following cytological confirmation of lymphoma, we subjected genomic DNA from the biopsies to targeted next generation sequencing (NGS), using a panel containing 126 genes (3,435 amplicons), which was modified from that of the NCI MATCH Trial. Using a validated bioinformatics pipeline, we assessed for the presence of actionable mutations and copy number alterations.

Results: In all four small-volume, intraocular liquid biopsies, we obtained sufficient genomic DNA for analysis. Using NGS, we found targetable heterozygous gain-of-function mutations in the MYD88 oncogene, and confirmed in our cohort the presence the L265 mutations, previously described using PCR-based assays. For the first time in VRL, we also identified the MYD88 S243N mutation. We also identified two-copy copy number losses in the tumor suppressor CDK2NA in all four cases, and one copy loss of the tumor suppressor PTEN in one sample. In one case, in which vitreous biopsies were originally read as cytologically negative, but which was confirmed as lymphoma when a lesion appeared in the brain two years later, our NGS-based approach detected tumoral DNA in the banked, original liquid biopsy.

Conclusions: We performed the first systematic exploration of the actionable cancer genome in VRL. Our NGS-based approach identified exploitable genomic alterations in all cases, such as gain-of-function MYD88 oncogene mutations and loss of the tumor suppressor CDK2NA, and thus illuminates new routes to biologically targeted therapies for VRL, a cancer with a dismal prognosis. Bioinformatics analysis yields candidate point mutations and copy number alterations that potentially drive tumor growth and development in vitreoretinal lymphomas. Potentially actionable therapeutic targets are prioritized and reported.

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Diagnostic imaging of retinoblastoma in pediatric patients with a novel 1050nm optical coherence tomography clinical system

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\textbf{Purpose:} Reliable differentiation in real time between the vital tumor tissue and benign mass is the main challenge for treatment of retinoblastoma (RB), a retinal malignancy occurring in infancy. Our ongoing observational clinical study uses the novel optical coherence tomography (OCT) imaging system specifically developed for pediatric retinoblastoma patients under anesthesia. We demonstrate that the 3-D mapping of tissue structure at the 8-15 \(\mu \text{m}\) resolution of OCT increases sensitivity for detection of vital tumor tissue in-vivo and in real time.

\textbf{Methods:} The 0-4 year old RB patients were recruited over the period of 2 years from the 10-15 new annual cases in the Netherlands. The standard diagnostics, treatment, and follow-up monitoring were performed in parallel with OCT imaging.

OCT is a non-invasive optical modality that produces cross sectional images of tissue up to a 2mm in depth. The 3-D visualization is a powerful tool for assessment of various retinal abnormalities and effects from treatment including scar tissue, calcification, benign masses, and retinopathy. Our novel imaging system with a handheld scanner is implemented for children in supine position under inhalation anesthesia.

\textbf{Results:} The 3-D mapping of retinal tissue morphology improves diagnosis and helps solve ambiguous cases as will be illustrated by the patient data. The examples from 3 different patients are shown in the figures below. The first two cases (Fig 1) are the vital RB tumors both after (left) and before (right) laser treatment. They are characterized by dense uniform masses with sideways extension. Calcified spots are present in the receding tumor in the laser-treated case. The inactive regression type 2 lesion with retinal layers wrapping around it (Figure 2) has a different shape with volume unchanged in follow-up imaging. In standard funduscopy exam the three cases look similar in en-face images, while ultrasonography does not provide sufficient resolution to distinguish between them.

\textbf{Conclusions:} We demonstrate the increased real-time diagnostic sensitivity for retinoblastoma patients by imaging the 3D morphology of retina at OCT resolution, while in some cases follow-up evaluation is still needed to validate the initial diagnosis.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{OCT_images.png}
\caption{OCT images of RB tumors from 2 patients: laser-treated (left), and before laser treatment (right). Scale bars: 0.25mm}
\end{figure}
that induces Vhl deletion in angioblast-derived cells. Fundoscopy was done monthly to detect retinal lesions. Fluorescein angiography (FA) was performed on affected mice. All retinas were analyzed by histology at 4 months of age. Genome typing of the Vhl conditional KO allele was conducted in retinal lesions using microdissection, nest-PCR and Sanger sequencing.

Results: About 64% (18/28) of the transgenic mice exhibited various retinal vascular defects following induction. Affected mice demonstrated retinal vascular lesions that were variably associated with prominent vessels, anomalous capillary networks, hemorrhage, exudates, and localized fibrosis. FA revealed vascular leakage from the lesions. Histological analyses showed RCH-like lesions of tortuous, dilated vessels surrounded by “tumorlet” cells, isolated foamy stromal cells, and glia, classically found in RCH. Vhl LOH was detected in the tumor-like area as verified by sequencing.

Conclusions: This is the first demonstration of VHL-associated RCH in a transgenic mouse model. This model may be useful for studying RCH pathogenesis, including HIF-dependent and HIF-independent pathways, and for testing potential therapies.

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CXCR4 expression in an intraocular melanoma mouse model with hepatic metastases

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Purpose: Tumor cell CXCR4 expression is an associated risk factor for metastasis. The purpose of this study is to characterize CXCR4 expression in intraocular melanoma and its hepatic metastases in a mouse model and image hepatic metastases using MRI with a novel designed CXCR4-targeted contrast agent (ProCA32-CXCR4).

Methods: 5 different human uveal melanoma cell lines, M20-09-196, M20-07-070, 92.1, OMM3 and Mel290 were evaluated by flow cytometry for the level of CXCR4. Nude mice were inoculated intraocularly with these 5 cell lines. CXCR4 expression was analyzed by immunohistochemistry (IHC) in ocular and hepatic tissues. To understand whether host deficient immune system affect CXCR4 expression in tumors, ocular and hepatic tissues from strains of mice (C57BL/6 with or without anti-asialo GM1, DBA/2J, DBA/2J, Gpmnb- and Bxd102) inoculated in our well-established intraocular mouse melanoma model system were evaluated by IHC as well. The abdomen of mice inoculated M20-09-196 was scanned by MRI at 30 minutes, 3, 24, 48 hours after the injection of ProCA32-CXCR4 by tail vein, and before the injection.

Results: Flow cytometry showed differential expression of CXCR4 (percentage): 11.85% of 92.1, 30.7% of M20-07-070, 60.95% of OMM3, 61.85% of Mel290 and 91.0% of M20-09-196. The intraocular melanomas and their hepatic metastases expressed CXCR4 in all strains of mice tested. There were more hepatic metastasis in the mice inoculated by M20-09-196 (a cell line with a high level of CXCR4, 34.33±11.30) than the other cell lines tested (P<0.01). MRI images showed hyper-intensity in hepatic metastases using the T1 weighted spin echo sequence with ProCA32-CXCR4 in vivo, comparing to homogeneity of intensity in liver under pre-scan.

Conclusions: All mouse intraocular melanoma models and their hepatic metastases in this study expressed CXCR4, regardless of BAP1 status, host immune status or the level of CXCR4 expression in vitro. While the number of hepatic metastasis is positively correlated with the level of CXCR4 in vitro, MRI with a novel designed CXCR4-targeted contrast agent confirmed overexpression of CXCR4 in hepatic metastases and is capable to detect hepatic metastases in small size. MRI with the novel designed targeted contrast agent might provide the guide to select therapeutic agents.

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Amyloid beta deposits in ex vivo retinas correlate with the severity of Alzheimer’s brain pathology

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Purpose: Alzheimer’s disease (AD) is a neurodegenerative disease with characteristic deposition of amyloid beta (Aβ) in the brain. The retina, in which Aβ deposits have been detected in vivo, is optically accessible. Here we compare ex vivo Aβ neuropathology of the brain and eye at time of death.

Methods: Eyes and brains were obtained in compliance with the Declaration of Helsinki from healthy subjects and patients diagnosed with AD. One eye was fixed in 4% paraformaldehyde or 10% formalin. The retina was stained with 0.1% Thioflavin-S and 0.1% Thioflavin-T and examined using a Nikon transmission (FITC) microscope, equipped with a polarimeter. Aβ deposits were counted using fluorescence and polarimetry. Quadrants not examined, were assigned a number of Aβ deposits equal to the average number of Aβ deposits in the other quadrants. Brains were examined by a neuropathologist and the severity of AD pathology determined according to NIA guidelines based on assessment of neuritic senile plaque numbers (CERAD), anatomic distribution of Aβ deposits (Thal phase, TP), anatomic distribution of tau-immunoreactive neurofibrillary tangles (Braak stage, BS), and a cumulative score of the severity of AD pathology (CSS). The number of deposits found in the anterior layers of the retina with a signal in both fluorescence and polarimetry were then compared to these measures of AD brain pathology.

Results: There were no Aβ deposits in the retina of the individual whose brain showed no AD pathology. For individuals with a high level of AD brain pathology (n=6), there were more retinal Aβ deposits than in the individual with an intermediate level of AD brain pathology. The Spearman’s r (p) between number of retinal
Aβ deposits and CSS as well as TP of AD brain pathology were significant, but insignificant for CERAD as well as for BS. Gamma (γ) agreed with ρ for CSS and TP (γ=1 strong relationship) and BS (γ=0.33 weak relationship), but not for CERAD (γ=0.76 moderate relationship).

**Conclusions:** These Aβ deposits in the retina would be large enough to be resolved in vivo. In this initial sample, the number of retinal Aβ deposits correlates both to a cumulative score of AD brain pathology and to a measure of brain amyloid pathology. There was a weak relationship with neurofibrillary tangles. Thus in vivo retinal imaging of Aβ deposits is a promising diagnostic of AD brain pathology.

**Methods:** Three groups of participants were examined by Dynamic Vessel Analyzer (IMEDOS Systems, Jena, Germany): 11 patients, 76.2 (72.5 – 80.0) y.o. [median (1st quartile – 3rd quartile)], with mild-to-moderate dementia due to probable AD fulfilling the standard diagnostic criteria (ADD); 20 patients, 69.2 (63.6 – 72.0) y.o. with mild cognitive impairment (MCI) due to AD, and 14 anamnestic healthy control subjects 67.1 (58.5 – 70.8) y.o. without cognitive impairment (HC). Oscillatory temporal changes of retinal vessel diameters were assessed during 40 s and were evaluated using mathematical signal analysis.

**Results:** The relative to the vessel diameter magnitude of arterial oscillations was higher in ADD: 6.5% (6.1% – 7.5%) vs. HC: 4.4% (3.6% – 5.9%), p<0.01. Temporal shift between arterial and venous pulsations was different in ADD: 0.00 (-0.44 – 0.16) s vs. HC: 0.14 (0.01 – 0.69) s, p<0.05. Power spectra of the temporal curves differed between the groups especially within low frequency range: 0 – 0.2 Hz. In arteries at very low frequencies < 0.05 Hz HC oscillations, characterized by the normalized area under the power spectrum, prevailed over ADD and MCI oscillations. At moderate low frequencies (0.05 – 0.2 Hz) which correspond to lymphatic vessels’ pulsations, ADD and most MCI oscillations prevailed over HC oscillations.

**Conclusions:** Functional and morphological alterations in the retinal vessels in ADD are shown using a non-invasive in-vivo technique. The results are consistent with the amyloid clearance hypothesis of substrates being cleared from the brain along paravascular pathways due to vascular pulsation. The emphasized retinal arterial pulsation at moderate low frequencies in ADD and MCI group would be compatible with the view of compensatory overregulation in AD. Dynamic retinal vessel analysis could allow to reveal the etiology of AD and to contribute to its additional diagnostic characterization.

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**Dynamic retinal arterial and venous oscillations are changed in Alzheimer’s disease**

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**Purpose:** Vascular risk factors contribute to the development of Alzheimer’s disease (AD). Retinal vessels are similar to cerebral vessels in their structure and function. We demonstrated previously that non-stimulated temporal retinal arterial and venous oscillations (pulsations, vasomotions) are changed in healthy volunteers with age, in primary open angle glaucoma and in diabetes mellitus type 1. Whether this dynamic retinal vessel behavior is altered in AD and related to clinical severity is investigated.

**Methods:** Three groups of participants were examined by Dynamic Vessel Analyzer (IMEDOS Systems, Jena, Germany): 11 patients, 76.2 (72.5 – 80.0) y.o. [median (1st quartile – 3rd quartile)], with mild-to-moderate dementia due to probable AD fulfilling the standard diagnostic criteria (ADD); 20 patients, 69.2 (63.6 – 72.0) y.o. with mild cognitive impairment (MCI) due to AD, and 14 anamnestic healthy control subjects 67.1 (58.5 – 70.8) y.o. without cognitive impairment (HC). Oscillatory temporal changes of retinal vessel diameters were assessed during 40 s and were evaluated using mathematical signal analysis.

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**Conclusions:** Functional and morphological alterations in the retinal vessels in ADD are shown using a non-invasive in-vivo technique. The results are consistent with the amyloid clearance hypothesis of substrates being cleared from the brain along paravascular pathways due to vascular pulsation. The emphasized retinal arterial pulsation at moderate low frequencies in ADD and MCI group would be compatible with the view of compensatory overregulation in AD. Dynamic retinal vessel analysis could allow to reveal the etiology of AD and to contribute to its additional diagnostic characterization.

**Commercial Relationships:** Konstantin E. Kotliar, None; Chiristine Hauser, None; Marion Ortner, None; Ines Lanzl, None; Christoph Schmaderer, None; Timo Grimmer, None

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