Molecular Pathology Abstracts, 12/17

Editors: Donna E. Hansel, MD, PhD, chief, Division of Anatomic Pathology, and professor, Department of Pathology, University of California, San Diego; John A. Thorson, MD, PhD, associate professor of pathology, director of the Clinical Genomics Laboratory, Center for Advanced Laboratory Medicine, UCSD; Sarah S. Murray, PhD, associate professor, Department of Pathology, and director of genomic technologies, Center for Advanced Laboratory Medicine, UCSD; and James Solomon, MD, PhD, resident, Department of Pathology, UCSD.

Refining subgroups of pediatric gliomas using molecular markers

Pediatric high-grade and diffuse intrinsic pontine gliomas are a rare and heterogeneous group of tumors that show diverse histology, location, and prognosis. Although little was known regarding the development of these tumors, recent genomic studies have begun to elucidate their biological underpinnings. The authors conducted a study to further understanding of such gliomas by breaking them into subgroups. They analyzed 20 publicly available datasets containing 910 pediatric glioma cases, including rare pediatric high-grade gliomas (World Health Organization grade III or IV) and diffuse intrinsic pontine gliomas (grades II–IV). They also included in their analysis 157 unpublished cases of pediatric high-grade glioma and diffuse intrinsic pontine glioma. The majority of the samples were collected pretreatment and had varying amounts of genomic data available. Several groups of gliomas emerged that had distinct locations, survival rates, and demographics based on specific genomic alterations. The first subgroup investigated was derived from 903 samples that had mutation data for histone H3 genes. Tumors with H3.3G34R/V mutations (encoded by genes *H3F3A* and *H3F3B*) almost always occurred in the cerebral hemispheres, particularly the parietal and temporal lobes, and were found in adolescents and young adults. This subgroup had longer overall survival (median, 18 months) compared with other H3-defined subgroups. A second subgroup of tumors with H3.3K27M mutations was spread throughout the midline and pons regions of the brain. The mutations were associated with a significantly shorter time to death from disease (median, 11 months). A third subgroup had K27M mutations in either H3.1 (encoded by 10 genes clustered on chromosome 6) or H3.2 (encoded by genes *HIST2H3A, HIST2H3C*, and *HIST2H3D*). Those tumors occurred predominately in the pons region of the brain, affected a younger age group (median, five years), and were associated with longer overall survival (median, 15 months) than tumors with the same mutation in H3.3 genes. A fourth subgroup, with *IDH1* R132 mutation status available, was also investigated (n=640). Tumors with the *IDH1* mutation were restricted to the forebrain, occurred in an older group of patients (median, 17 years), and were associated with longer overall survival (59 percent; two-year survival). Finally, a subgroup of cases with absence of histone H3 or *IDH1* mutations was investigated, and a subset of largely hemispheric tumors emerged that included pleomorphic xanthoastrocytoma (PXA)-like and low-grade glioma-like subgroups. This subset was driven by *BRAF* V600E, *NF1* mutations, or fusions of receptor tyrosine kinases, including *MET, FGFR2, NTRK2,* and *NTRK3*. This group had the best overall survival rate (median, 63 months). With regard to copy number abnormalities, the study found recurrent gains of 1q and losses of 13q and 14q, as well as a large proportion of tumors with little or no copy number abnormalities. Loss of 17p (encompassing *TP53*) was also observed and conferred a shorter overall survival in all tumor cases, regardless of location or subgroup. The authors observed focal amplifications, including 4q12 (*PDGRFA/KIT/KDR*), 2p24.3 (*MYCN/ID2*), 7p11.2 (*EGFR*), 7q21.2 (*CDK6*), and 7q31.2 (*MET*), as well as focal loss of 9p21.3 (*CDKN2A/CDKN2B*). In general, amplifications conferred shorter overall survival, and CDKN2A/CDKN2B deletion conferred a better prognosis. This study highlights the diversity of pediatric gliomas, as well as the utility of profiling both genomic sequence and copy number variants to define subgroups that have implications regarding diagnosis, prognosis, and potential therapeutic options.

Mackay A, Burford A, Carvalho D, et al. Integrated molecular meta-analysis of 1,000 pediatric high-grade and diffuse intrinsic pontine glioma. *Cancer Cell.* 2017;32(4):520–537. e1–e5. doi:10.1016/j.ccell.2017.08.017.

Correspondence: Dr. Chris Jones at chris.jones@icr.ac.uk

[hr]

Promise of using cfDNA for retinoblastoma tumor biopsy

Retinoblastoma, a rare eye tumor that typically occurs early in childhood, is often curable when diagnosed early. The genetics of retinoblastoma follow the classic “two-hit” tumor-suppressor gene model, which suggests that both copies of a tumor-suppressor gene are lost, usually through mutation or deletion, thus eliminating the controls on tumor development. This may occur through a combination of an inherited alteration paired with a somatic alteration or through two somatic alterations. However, identification of somatic *RB1* alterations has been limited due to the inability to biopsy specimens of the eye since the biopsy procedure may result in tumor seeding outside the eye. The authors conducted a study in which they provided evidence that somatic genomic alterations may be detected using cell-free (cf) DNA collected from the aqueous humor, a watery fluid that maintains intraocular pressure. The study evaluated genome-wide somatic copy-number alterations and RB1 mutations through next-generation sequencing using specimens from three retinoblastoma patients, including cfDNA derived from six aqueous humor samples and tumor tissue derived from three eyes with retinoblastoma. The first patient in the study had unilateral retinoblastoma, and analysis of both an aqueous humor cfDNA sample and a tumor sample from the patient’s eye revealed copy number gains in 1q and 6p, common chromosomal abnormalities in retinoblastoma. The second patient also had unilateral retinoblastoma but carried a known constitutional deletion of 13q that encompassed the *RB1* gene. However, results from the paired aqueous humor cfDNA and tumor specimen in this patient did not align completely. Both specimens had gains of 1q and 6p, and unique copy number alterations were observed in the specimens as well, with most occurring in the aqueous humor specimen. The authors proposed that given the second patient’s predisposition to retinoblastoma due to the “first hit” from the constitutional 13q deletion, multiple clonally distinct tumors developed in the eye, and the additional copy number alterations observed in the aqueous humor could represent a composite of the multiple tumor clones, differing from the single tumor clone assayed from the enucleation specimen. Finally, a third patient with bilateral disease had multiple aqueous humor samplings over different time points, coinciding with multiple intraocular injections of chemotherapy. Common retinoblastoma chromosome abnormalities and copy number changes were found in both tumor and aqueous humor cfDNA, resulting in a more complex genomic profile than that observed in the other two cases. This complex genomic profile was proposed to result from a mosaic *RB1* truncating mutation detected in the DNA derived from peripheral blood, which may place this patient at higher risk for retinoblastoma tumor development. This study, although small, provides insights into the use of cfDNA from aqueous humor to profile copy number alterations as a surrogate to genomic profiling of the retinoblastoma tumor directly. The ability to do this less-invasive sampling, which poses less risk for tumor seeding, can provide insights into the complexity of genomic alterations and potentially inform personalized therapeutic or clinical treatment decisions, including eye enucleation. Although larger studies are needed to more broadly assess the validity of this approach for profiling retinoblastoma tumors, this study presents a novel and promising molecular profiling paradigm using cfDNA.

Berry JL, Xu L, Murphree AL, et al. Potential of aqueous humor as a surrogate tumor biopsy for retinoblastoma [published online ahead of print October 12, 2017]. *JAMA Ophthalmol.* doi:10.1001/jamaophthalmol.2017.4097.

Correspondence: Dr. Jesse L. Berry at jesse.berry@med.usc.edu