An integrated approach to identifying clinically relevant targets in pediatric gliomas

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High-grade gliomas (HGGs) are categorized by WHO as grade III–IV glial malignancies and account for roughly 15–20% of all pediatric CNS tumors [1]. For pediatric patients diagnosed with supratentorial HGGs, the 2-year survival rates range from 10 to 30%. The prognosis for patients with diffuse brainstem gliomas is even more dismal, as less than 10% will survive more than 2 years after diagnosis [1]. The current treatment for newly diagnosed pediatric HGGs is safe maximal surgical resection and radiotherapy. The addition of chemotherapy to this regimen is debatable, reflecting the lack of consensus among pediatric neuro-oncologists regarding the optimal protocol/treatment plan for newly diagnosed patients [2]. Despite these efforts, marginal impact has been made on the overall survival rates of these patients. This begs the question: ‘how do we change the way in which we view and treat pediatric HGGs?’

To fully understand the complexities of pediatric HGGs and subsequently identify therapeutic vulnerabilities, we must first understand the full molecular, cellular and

physiological context of these malignancies. While it has long been thought that both pediatric and adult gliomas are the same disease affecting different patient populations, recently, multiple groups including the Canadian Paediatric Cancer Genome Consortium and the St Jude Children’s Research Hospital–Washington University in St Louis Pediatric Cancer Genome Project have challenged this notion by revealing striking differences in the mutation spectrum and frequencies between pediatric and adult HGGs [3–11]. The use of high-resolution genomic approaches has uncovered potential therapeutic targets for pediatric gliomas such as *PDGFRA*, *MET* and *IGF1R* (recurrent focal amplification in HGGs and pontine gliomas) [7,10,12], *BRAF* and *CDK2NA* (*BRAF*-V600E mutations with concomitant *CDKN2A* loss in a subset of malignant astrocytomas) [11] and *FGFR1* (intragenic duplications of the tyrosine kinase domain in low-grade gliomas) [3].

Alone, the identification and characterization of the somatic DNA alterations is insufficient. Pediatric HGGs harbor

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histone H3 mutations and display unique DNA methylation profiles, implying that there are underlying epigenetic mechanisms driving tumor progression [4–6]. Importantly, the distinct epigenome present in pediatric HGGs likely drives a transcriptional profile that is markedly different from that of adults, thus prompting several research teams to perform in-depth transcriptome analyses to uncover potential gene targets and/or cellular processes. To date, the standard method for analyzing global gene transcription has been gene expression arrays. This technology is informative but incomplete due to the limitation of genes spotted on the arrays. However, next-generation RNA sequencing is a more comprehensive and quantitative approach to transcriptome profiling. This method enables the capture of a variety of RNA species that are not accounted for on standard gene expression arrays, including fusion transcripts, splice variants, miRNAs and lncRNAs. An emerging concept in the field of cancer biology is that lncRNAs have functional significance in various malignancies. For instance, the lncRNA *ANRIL* (also known as *CDKN2B-AS*) contributes to the repression of the *INK4a/INK4b/ARF* locus, while expression of the lncRNA *HOTAIR* is associated with disease progression and poor outcome in numerous cancers [13]. Intriguingly, both of these lncRNAs interact with lysine 27 on histone H3, which is mutated in 78% of pediatric HGGs [4]. Currently, novel inhibitors of histone methyltransferases and histone deacetylases are in various stages of development for use in the clinic. The therapeutic efficacy of these new drugs in treating pediatric HGGs is not yet known. Irrespective of their clinical success, these drugs should be considered investigational tools to probe the relevant epigenetic mechanisms that drive pediatric HGGs.

To date, the biggest hindrance to pediatric glioma research has been the availability of patient material for experimentation. This is especially true for tumors upon which biopsies are not routinely performed, such as diffuse intrinsic pontine gliomas. Implementation of clinical protocols allowing for the procurement and subsequent propagation of patient-derived biopsy and autopsy material has proven beneficial to circumventing this hurdle. The tumor cells can be cultured *in vitro* as well as *in vivo* via intracranial implantation, allowing for targeted dissection of signaling pathways, target validation studies, pharmacokinetic/pharmacodynamic drug

studies, tumor–host interaction studies and a myriad of other investigations. One advantage to this approach, with regard to the study and the development of rational targets and therapeutics, is the ability of patient-derived material to recapitulate the genetic intratumoral heterogeneity that has been identified in HGGs [7]. In addition, patient-derived xenografts can reveal subclonal populations of cells that exhibit different degrees of tumorigenicity and therapeutic sensitivity. Furthermore, the material obtained from the patients' tumors reflects the natural progression of disease and the histology of patient-derived xenografts often mimics many of the features present in the human tumor of origin [14]. The ability to model these aspects of pediatric HGGs allows for the development of rational strategies on how to effectively pursue multiple targets within tumor cell populations that are clinically relevant. One limitation of using these models is that the patient-derived xenografts are studied in immunocompromised animals. Although the proper anatomical microenvironmental stimuli cannot be accurately recapitulated in orthotopic xenografts, with brainstem glioma models being particularly problematic and difficult, these models are crucial to the preclinical assessment and validation of novel targets, as well as to the investigation of US FDA-approved drugs for off-label use.

Furthermore, various genetically engineered mouse (GEM) models have been developed to interrogate specific signaling pathways known to be deregulated in pediatric and adult gliomas [15]. In the context of identifying clinically relevant HGG targets, these models have proven to be valuable in studying the genetics underlying disease progression. The gliomagenic nature of specific alterations to candidate signaling pathways can be elucidated using GEM models as well as the mechanisms and effects of selective pharmacologic pathway inhibitors. Moreover, genetically engineered mice allow for the differences between mutations or pathways required for glioma formation versus glioma progression to be outlined. Importantly, some of these genetically engineered models develop HGGs with strikingly similar histopathological features to the human disease [15,16]. As with all models, the use of genetically engineered mice for target discovery and/or validation has its limitations. These GEM models are costly to develop and maintain, and require a significant amount of time for both the establishment of

the mice and the development and characterization of the tumor. Single mutations of interest are often inadequate drivers of the disease and must be combined with additional mutations to generate the desired pathology, highlighting the discordance between genotypic and phenotypic mouse models of glioma [15]. In addition, mutations have to be introduced into specific cell types and are subsequently expressed at precise times to generate the desired glioma. This is increasingly challenging as the cell(s) of origin for both pediatric and adult gliomas is subject to ongoing investigation [15,17].

Unraveling the intricacies of signal transduction networks is a daunting task in non-neoplastic tissues and cells. The complexity of this undertaking increases dramatically in cancer. In comparison with the adult disease, pediatric HGGs contain fewer alterations in the three core glioma signaling pathways assigned by The Cancer Genome Atlas (receptor tyrosine kinase/phosphoinositide 3-kinase, Rb and P53 pathways) [8]. This suggests that pediatric HGGs use alternative signaling networks. Analysis of the phosphoproteome is an unbiased method to identify and investigate these interactions. Proteins extracted from archived formalin-fixed paraffin-embedded patient tumors, freshly procured tumor material or patient-derived xenografts can be selectively enriched for phosphoproteins (phosphorylated serine, threonine, tyrosine and histidine residues) and subsequently analyzed by mass spectrometry [18]. This quantitative approach is much more robust than other biochemical-based assays, albeit more time consuming and costly. In addition to the discovery of novel signaling pathways, mass spectrometry can also be used to assess changes in tumor metabolism in response to different therapies [19]. Alternatively, commercially available reverse-phase protein arrays, also known as antibody arrays, can be used as a cheaper and less comprehensive surrogate. These arrays contain

several hundred different phosphorylated and unphosphorylated antibodies generated against numerous components of multiple signaling and transcription factor networks. Similar to gene expression arrays, the data obtained is dependent on how many proteins are represented on the array as well as the quality and exclusivity of the antibodies. In addition to identifying pathways that drive pediatric gliomagenesis, either of these tools can be used to monitor changes in multiple signaling pathways in response to treatment and elucidate compensatory mechanisms that may drive resistance.

The desperate need for novel methods to confront pediatric HGGs is apparent. This sentiment is shared between patients and their families, scientists, and clinicians. In the USA, the Creating Hope Act was recently implemented to incentivize the pharmaceutical industry to develop new therapies for rare pediatric diseases [20]. This act, along with high-resolution genomics, patient-derived xenografts, genotypic and phenotypic animal models, and proteomics, comprises the battery of tools and methods currently available to combat pediatric HGGs. These resources must be leveraged collectively and deliberately to put forth a more comprehensive approach that furthers our understanding of the signaling pathways and cellular processes vital to the establishment, maintenance, progression and/or recurrence of pediatric HGGs.

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References

- Broniscer A, Gajjar A. Supratentorial high-grade astrocytoma and diffuse brainstem glioma: two challenges for the pediatric oncologist. *Oncologist* 9(2), 197–206 (2004).
- Fangusaro J, Warren KE. Unclear standard of care for pediatric high grade glioma patients. *J. Neurooncol.* doi:10.1007/s11060-013-1104-8 (2013) (Epub ahead of print).
- Zhang J, Wu G, Miller CP *et al.* Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. *Nat. Genet.* 45, 602–612 (2013).
- Wu G, Broniscer A, McEachron TA *et al.* Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat. Genet.* 44(3), 251–253 (2012).
- Schwartzentruber J, Korshunov A, Liu X-Y *et al.* Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature* 482(7384), 226–231 (2012).
- Sturm D, Witt H, Hovestadt V *et al.* Hotspot mutations in *H3F3A* and *IDH1* define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell* 22(4), 425–437 (2012).
- Paugh BS, Broniscer A, Qu C *et al.* Genome-wide analyses identify recurrent amplifications of receptor tyrosine kinases and

- cell-cycle regulatory genes in diffuse intrinsic pontine glioma. *J. Clin. Oncol.* 29(30), 3999–4006 (2011).
- 8 Bax DA, Mackay A, Little SE *et al.* A distinct spectrum of copy number aberrations in pediatric high-grade gliomas. *Clin. Cancer Res.* 16(13), 3368–3377 (2010).
- 9 Qu H-Q, Jacob K, Fatet S *et al.* Genome-wide profiling using single-nucleotide polymorphism arrays identifies novel chromosomal imbalances in pediatric glioblastomas. *Neuro Oncol.* 12(2), 153–163 (2010).
- 10 Paugh BS, Qu C, Jones C *et al.* Integrated molecular genetic profiling of pediatric high-grade gliomas reveals key differences with the adult disease. *J. Clin. Oncol.* 28(18), 3061–3068 (2010).
- 11 Schiffman JD, Hodgson JG, VandenBerg SR *et al.* Oncogenic *BRAF* mutation with *CDKN2A* inactivation is characteristic of a subset of pediatric malignant astrocytomas. *Cancer Res.* 70(2), 512–519 (2010).
- 12 Bielen A, Perryman L, Box GM *et al.* Enhanced efficacy of IGF1R inhibition in pediatric glioblastoma by combinatorial targeting of PDGFR α/β . *Mol. Cancer Ther.* 10(8), 1407–1418 (2011).
- 13 Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. *RNA Biol.* 9(6), 703–719 (2012).
- 14 Tentler JJ, Tan AC, Weekes CD *et al.* Patient-derived tumour xenografts as models for oncology drug development. *Nat. Rev. Clin. Oncol.* 9(6), 338–350 (2012).
- 15 Chow LML, Baker SJ. Capturing the molecular and biological diversity of high-grade astrocytoma in genetically engineered mouse models. *Oncotarget* 3(1), 67–77 (2012).
- 16 Rankin SL, Zhu G, Baker SJ. Insights gained from modelling high-grade glioma in the mouse. *Neuropathol. Appl. Neurobiol.* 38(3), 254–270 (2012).
- 17 Chen J, McKay RM, Parada LF. Malignant glioma: lessons from genomics, mouse models, and stem cells. *Cell* 149(1), 36–47 (2012).
- 18 Yue X-S, Hummon AB. Mass spectrometry-based phosphoproteomics in cancer research. *Front. Biol.* 7(6), 566–586 (2012).
- 19 O'Brien P, Lee M, Spilker M *et al.* Monitoring metabolic responses to chemotherapy in single cells and tumors using nanostructure-initiator mass spectrometry (NIMS) imaging. *Cancer Metab.* 1(1), 4 (2013).
- 20 Connor E, Cure P. 'Creating hope' and other incentives for drug development for children. *Sci. Transl Med.* 3(66), 66cm1 (2011).