Unlocking the translational potential of circulating nucleosomes for liquid biopsy in diffuse intrinsic pontine glioma

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“It is time we shine a light on this deadly disease.”

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Diffuse intrinsic pontine glioma (DIPG) is a devastating pediatric brain tumor for which curative treatment remains elusive, and optimal management strategies other than radiation therapy are ineffective. This brain tumor constitutes one of the leading causes of cancer-related morbidity and mortality in children, with a median survival of 12 months despite best standard of care currently available [1]. As such, there is a growing and desperate need to develop novel diagnostic and therapeutic platforms to treat this deadly disease.

Recent advances in our understanding of this disease have uncovered the majority of DIPG tumors have a histone *H3* gene mutation that most commonly results in an H3K27M substitution (histone 3 lysine for methionine at site 27 [H3K27M]) [2,3]. In the latest WHO Classification of Central Nervous System (CNS) Tumors in 2016, DIPG was subsequently reclassified as a diffuse midline glioma with H3K27M mutation [4]. This signaled the growing relevance of histone proteins in DIPG tumors, as well as their epigenetic changes, primarily the global loss of trimethylation at other wild-type H3K27 sites.

Biologically, the H3K27M mutation can occur in several *H3* genes; however, most commonly it occurs in the *H3F3A* and *HIST1H3B/C* genes, which encode for *H3* variants H3.3 and H3.1, respectively [2,5]. This histone mutation can result in significant epigenetic changes in trimethylation and acetylation at H3 lysine (K) sites, and phosphorylation at H3 serine (S) sites [6]. Universally, in H3K27M tumors, there is global loss of H3K27 trimethylation, which is likely to be one of the main drivers for tumorigenesis [5,7,8]. Other significant changes reported are more variable, and include increased trimethylation at H3K4 and H3K9 (H3K4/9), and changes to phosphorylation at H3S10 and H3S28 (H3S10/28) [7,9,10]. These changes are posited to directly affect the tumorigenic tendencies of DIPG cells in different ways, with decreased trimethylation at H3K27 alleviating repressed transcription of tumor cells, increased trimethylation at H3K4/9 activating transcription of tumor cells and alterations in H3S10/28 further modulating these important H3 lysine residues [6,11]. Collectively, these epigenetic changes likely create a unique biological signature for DIPG tumors that if identified, could potentially determine a molecular diagnosis based on histone profile.

The propensity for H3K27M tumors to occur in functionally eloquent and anatomically intricate areas, historically precluded surgical intervention. This, when coupled to its poor prognosis, lead leaders in the field to recommend a clinical-radiographic identification instead of tissue biopsy. Recently, the feasibility of tissue biopsy has become accepted as our understanding of this disease has increased, and a recent study has shown it to be generally safe in a large cohort [12]. However, the surgical risks of significant complications including hemorrhage within the brainstem leading to neurological deficits cannot be trivialized. Even when tissue can be obtained, whether or not there will be sufficient sample for diagnostic purposes cannot be guaranteed. As a result, there is a need for a tool or biomarker that can ideally provide molecular data in a noninvasive non-limited fashion to be utilized both for potential diagnosis and optimization of treatment choice and monitoring.

One possible solution for DIPG patients is molecular tumor biomarkers obtained from body biofluids [13]. A liquid biopsy is an attractive goal in DIPG care, for it circumvents the risk of major surgery, and is still able to obtain biologic information about the tumor. To this end, cell-free tumor DNA in cerebrospinal fluid (CSF) of DIPG patients has recently been reported to be detectable in small cohorts [14,15]. One potential application of this discovery is that the DNA can feasibly be analyzed to determine whether or not the characteristic H3K27M mutation is present or not. This highlights the significant diagnostic potential of biofluids in DIPG if the mutation and other biomarkers can be identified. With respect to CSF as a liquid biopsy target, it is not known if the DIPG tumor needs to make contact with the ventricular system to shed this nuclear cargo into the CSF space, which is not global in DIPG patients. In addition, obtaining CSF still requires an invasive procedure (lumbar puncture); therefore, CSF may not be the optimal liquid biopsy biofluid in these patients should another, less invasive, biofluid be obtainable.

One potential biofluid in DIPG is blood plasma, which although yet to be reported in the literature, is a logical target. Circulating nucleosomes, strands of DNA wrapped around a histone core, are readily detected in patients plasma [16]. In addition to H3K27M mutation status assessment of the DNA, epigenetic changes in trimethylation, acetylation and phosphorylation can be assessed from the histones in the nucleosome cores, making them a more informative target for DIPG biopsy than cell-free DNA alone. Preliminary evidence (not published) from our laboratory indicates that circulating nucleosomes in DIPG patient-derived plasma and xenograft models can be feasibly isolated and analyzed for trimethylation levels at H3K27, as well as H3K27M mutation presence. In addition, during a current Pediatric Neuro-Oncology Consortium trial in DIPG tumor patients, they were able to detect H3K27M cell-free DNA in the majority of these patients (currently unpublished).

Unlike CSF, DIPG tumors are universally in contact with the blood vasculature potentially making plasma more sensitive and specific. Once released, these units of genetic material circulate within the blood until they are excreted. A liquid biopsy would involve venipuncture for whole blood sampling, from which the blood plasma and the circulating nucleosomes can be separated using centrifugation, and then readily evaluated. Techniques to evaluate the trimethylation, acetylation and phosphorylation status of the histone proteins can vary from ELISA to liquid chromatography–mass spectrometry. The advantage of analyzing nucleosomes for DIPG tumors is that given they have DNA strands as well, the H3K27M status can also be concurrently detected by Sanger sequencing after PCR amplification.

In solid cancers of the colon and pancreas, epigenetic alterations, such as changes in trimethylation at H3K9 and H4K20, have been shown to be readily detected from circulating nucleosomes in the plasma of patients with a high degree of sensitivity to the primary tumor, suggesting this nuclear data are tumor-derived and indeed circulating [17,18]. Plasma histone profiling is both an exciting and novel avenue of research in DIPG given its unique and significant histone biology. If genetic information about DIPG can be obtained by means of blood plasma sampling, it will provide clinicians and researchers a more applicable, readily accessible modality to evaluate the tumor than all the options currently available.

It is anticipated that a unique histone profile in DIPG plasma samples will distinguish itself from tumor-free plasma samples. In the DIPG samples, based on the assumption of parallel results to that of tumor tissue analyses, the characteristic changes that could be expected would be decreased trimethylation at H3K27, increased trimethylation at H3K4/9 and increased phosphorylation at H3K10. As H3K27M mutation status can also feasibly be detected from the DNA strands of the circulating nucleosomes, collectively, this proposed liquid biopsy could identify a mutation and histone profile specific for DIPG tumors only, complementing, and perhaps one day superseding, the need for tissue biopsy for diagnosis.

Furthermore, given any detected histone profile of the tumor epigenome would be dynamic in nature as circulating nucleosomes are constantly shed, there is the distinct possibility that repeat nucleosome analyses can indicate changes in epigenetic *H3* post-translational modifications, for example, restoration of trimethylation at K27 during effective treatment. Currently, there exists multiple novel drugs in the preclinical and clinical phase for DIPG tumors, with no viable platform for molecular monitoring. Such drugs include histone demethylase and deacetylase inhibitors, which are known to modulate the epigenome of these tumors [19,20]. Therefore, it would not be out of the realm of possibility that a liquid biopsy in patients treated with these drugs will result in a different histone profile that corresponds to their molecular response over time. The potential of this is significant, for currently, there is no effective modality to monitor molecular response of DIPG tumors, which will complement the more macroscopic magnetic resonance imaging (MRI) surveillance currently in practice.

The potential for a liquid biopsy in the field of DIPG is significant, especially if the emerging characteristic epigenetic histone profile of these tumors can be validated in the bloodstream. Not only will this be a less-invasive procedure which is crucial in the care of pediatric patients, it is also repeatable and dynamic, potentially detecting epigenetic changes with therapy over time. It is currently unclear if epigenetic changes can predict superior or inferior outcomes, simply because to date there has been no modality to assess epigenetic changes in DIPG patients at more than one time point (tissue biopsy is performed only once) – it is hypothesized that restoration of trimethylation at H3K27 could be one such change that could indicate a favorable prognosis or response.

If novel therapies can be rapidly evaluated for sensitivity based on epigenetic response of these circulating nucleosomes, then clinicians can be better equipped to determine in shorter amounts of time if a certain therapy should be pursued, rather than rely solely on radiographic responses which can take months before any potential change is evident. Additionally, it is envisaged that if analysis of circulating nucleosomes can be optimized, it will facilitate the development of real-time diagnosis and monitoring, which could prove vital in this era of personalized medicine. From a surgical perspective, real-time diagnostics could revolutionize the way these tumors are approached, and open the door for more effective testing of novel therapies intra-operatively which currently cannot be justified at such speed with the diagnostic tools currently available, in other words, histopathology.

In summary, DIPG is a devastating brain tumor in children that continues to have a dismal prognosis. Molecularly, there is a lack of access to biological tumor-related samples. Therapeutically, there is a lack of tools and biomarkers to monitor disease progression and response. Establishing a liquid biopsy of circulating nucleosomes would not only overcome these limitations, but also serve as diagnostic and therapeutic monitoring modality that can evaluate the H3K27M mutation status and epigenetic histone changes of DIPG patients in a noninvasive and rapid fashion. These biomarkers have desirable applications in clinic which will better our understanding and treatment of this disease, and therefore warrant future investigation. It is time we shine a light on this deadly disease.

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References

1. Merchant TE, Pollack IF, Loeffler JS. Brain tumors across the age spectrum: biology, therapy, and late effects. *Seminars Radiation Oncol.* 20(1), 58–66 (2010).
2. 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).
3. Castel D, Philippe C, Kergrohen T *et al.* Transcriptomic and epigenetic profiling of ‘diffuse midline gliomas, H3 K27M-mutant’ discriminate two subgroups based on the type of histone H3 mutated and not supratentorial or infratentorial location. *Acta Neuropathol. Commun.* 6(1), 117 (2018).
4. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK. *WHO Classification of Tumours of the Central Nervous System, Revised. Fourth Edition.* IARC Press, Lyon, France (2016).
5. Castel D, Philippe C, Calmon R *et al.* Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. *Acta Neuropathol.* 130(6), 815–827 (2015).
6. Maury E, Hashizume R. Epigenetic modification in chromatin machinery and its deregulation in pediatric brain tumors: insight into epigenetic therapies. *Epigenetics* 12(5), 353–369 (2017).
7. Chan KM, Fang D, Gan H *et al.* The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev.* 27(9), 985–990 (2013).
8. Fang D, Gan H, Cheng L *et al.* H3.3K27M mutant proteins reprogram epigenome by sequestering the PRC2 complex to poised enhancers. *eLife* 1, 7 (2018).
9. Buczkowicz P, Bartels U, Bouffet E, Becher O, Hawkins C. Histopathological spectrum of paediatric diffuse intrinsic pontine glioma: diagnostic and therapeutic implications. *Acta Neuropathol.* 128(4), 573–581 (2014).

10. Pathak P, Jha P, Purkait S *et al.* Altered global histone-trimethylation code and H3F3A-ATRX mutation in pediatric GBM. *J. Neuro-oncol.* 121(3), 489–497 (2015).
11. Lau PNI, Cheung P. Histone code pathway involving H3 S28 phosphorylation and K27 acetylation activates transcription and antagonizes polycomb silencing. *Proc. Natl Acad. Sci. USA* 108(7), 2801 (2011).
12. Gupta N, Goumnerova LC, Manley P *et al.* Prospective feasibility and safety assessment of surgical biopsy for patients with newly diagnosed diffuse intrinsic pontine glioma. *Neuro-oncology* 20(11), 1547–1555 (2018).
13. Traylor JJ, Kuo JS. “Liquid biopsy” of circulating tumor DNA characterizes treatment response in pediatric patients diffuse midline glioma. *Neurosurgery* doi: 10.1093/neuros/nyz029 (2019) (Epub ahead of print).
14. Pan C, Diplas BH, Chen X *et al.* Molecular profiling of tumors of the brainstem by sequencing of CSF-derived circulating tumor DNA. *Acta Neuropathol.* 137(2), 297–306 (2019).
15. Huang TY, Piunti A, Lulla RR *et al.* Detection of histone *H3* mutations in cerebrospinal fluid-derived tumor DNA from children with diffuse midline glioma. *Acta Neuropathol. Commun.* 5(1), 28 (2017).
16. McAnena P, Brown JA, Kerin MJ. Circulating nucleosomes and nucleosome modifications as biomarkers in cancer. *Cancers* 9(1), 1–18 (2017).
17. Gezer U, Yoruker EE, Keskin M, Kulle CB, Dharuman Y, Holdenrieder S. Histone methylation marks on circulating nucleosomes as novel blood-based biomarker in colorectal cancer. *Internat. J. Mol. Sci.* 16(12), 29654–29662 (2015).
18. Syren P, Andersson R, Bauden M, Ansari D. Epigenetic alterations as biomarkers in pancreatic ductal adenocarcinoma. *Scandinavian J. Gastroenterolo.* 52(6–7), 668–673 (2017).
19. Wetmore C, Boyett J, Li S *et al.* Alisertib is active as single agent in recurrent atypical teratoid rhabdoid tumors in 4 children. *Neuro-oncology* 17(6), 882–888 (2015).
20. Hashizume R. Epigenetic targeted therapy for diffuse intrinsic pontine glioma. *Neurol. Medico-chirurgica* 57(7), 331–342 (2017).