Circulating histone signature of pediatric Diffuse Intrinsic Pontine Glioma (DIPG)

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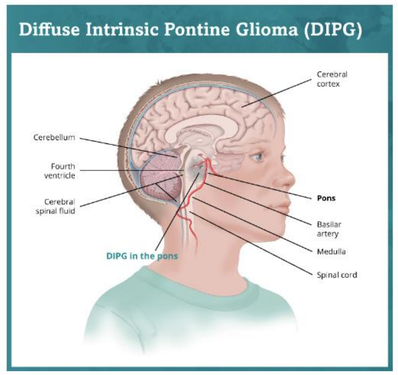
ABSTRACT

Introduction: Diffuse intrinsic pontine glioma (DIPG) is usually diagnosed when children are aged ten or below. It is a devastating and fatal disease with a median overall survival of less than 12 months after diagnosis. Radiological imaging is the gold standard for DIPG diagnosis while the use of invasive and risky biopsy focuses on the understanding its molecular biology, such as the histone H3K27M mutation, identified in ~30% of the cases. The urgent need to improve the survival encourages targeting biofluids such as cerebrospinal fluids (CSF) and blood plasma for optimizing molecular diagnoses in DIPG. Here, we propose a new, fast, imaging and epigenetics based approach to diagnose DIPG in the plasma of pediatric patients.

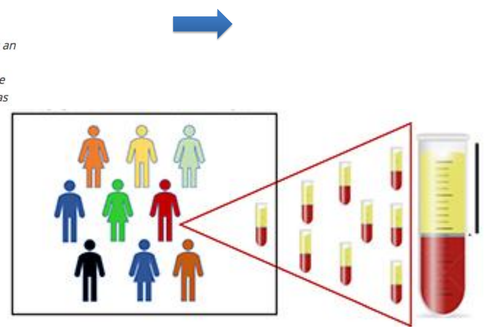
Material and Methods: a total of 20 healthy children (mean age 10.5] and 25 children diagnosed with DIPG (mean age 8.5) were recruited. 8/25 DIPG patients displayed histone H3K27M mutation. Individual histones, histone dimers and nucleosomes (histone tetramers) were assayed in serum samples by means of a new advanced flow cytometry ImageStream(X)-adapted method (1,2).

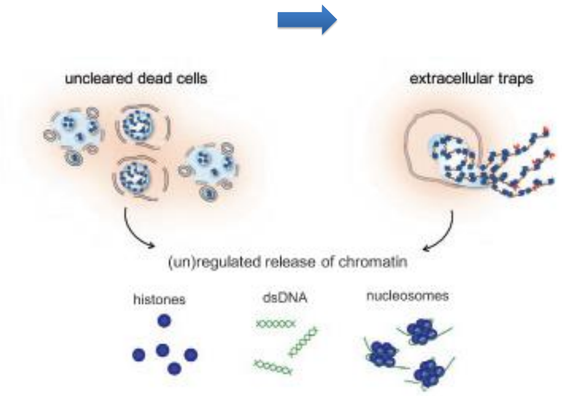
Results and Discussion: We implemented successfully a multi-channel flow methodology on ImageStream(X), to image single histone staining (H2A, H2B, H3, H4, macroH2A1.1 and macroH2A1.2). We report here a significant upregulation of histone dimers and tetramers (macroH2A1.1/H2B vs control: p-value<0.0001; macroH2A1.2/H2B vs control: p-value<0.0001; H2A/H2B vs control: p-value<0.0001; H3/H4 vs control: p-value =0.008; H2A/H2B/H3/H4 vs control: p-value<0.0001) and a significant downregulation of individual histones (H2B vs control: p-value<0.0001; H3 vs control: p-value<0.0001; H4 vs control: p-value<0.0001). Moreover, using a sample subset we show that individual histones and histone complexes are also detectable with a robust signal in the CSF of DIPG children.

Conclusion: In summary, we identified a new circulating histone signature able to discriminate the presence DIPG in children, using a rapid and non-invasive ImageStream(X)-based imaging technology. The patterns observed suggest the differential involvement of histone chaperone complexes in histone extracellular release in DIPG children plasma.



Diffuse intrinsic pontine glioma (DIPG) is an aggressive brain tumor. It begins in the brainstem in an area called the pons. The pons controls vital life functions as well as the nerves that control vision, hearing, speech, swallowing, and movement.





It combines the speed, sensitivity, and phenotyping abilities of flow cytometry with the detailed imagery and functional insights of microscopy

It allows comparison of levels and spatial associations for multiple fluorescent probes/signals.

Workflow. A total of 20 healthy children (mean age 10.5] and 25 children diagnosed with DIPG (mean age 8.5) were recruited. 8/25 DIPG patients displayed histone H3K27M mutation. Blood plasma Individual histones, histone dimers and nucleosomes (histone tetramers) were assayed in serum samples by means of a new advanced flow cytometry ImageStream(X)-adapted method.

Core histones	Histone variants
H2A, H2B, H3, H4	H3.1, H3.2, H3.3, CENPA, H2AZ, H2AX, macroH2A1.1, macroH2A1.2, macroH2A2, H2A.Bbd, TH2B, H2BFW, SubH2BV, H2BL1, H2BL2

Figure 1. There are 19 histone species, between core histones and histone variants. We measured the plasma concentration of all core histones + histone variants macroH2A1.1/macroH2A1.2

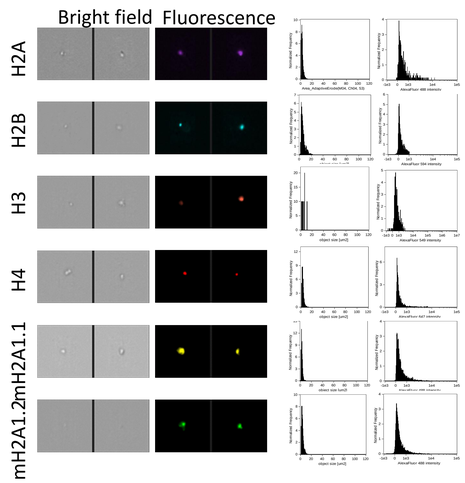


Figure 2. Multiplex histone imaging in DIPG patients plasma. Representative images.

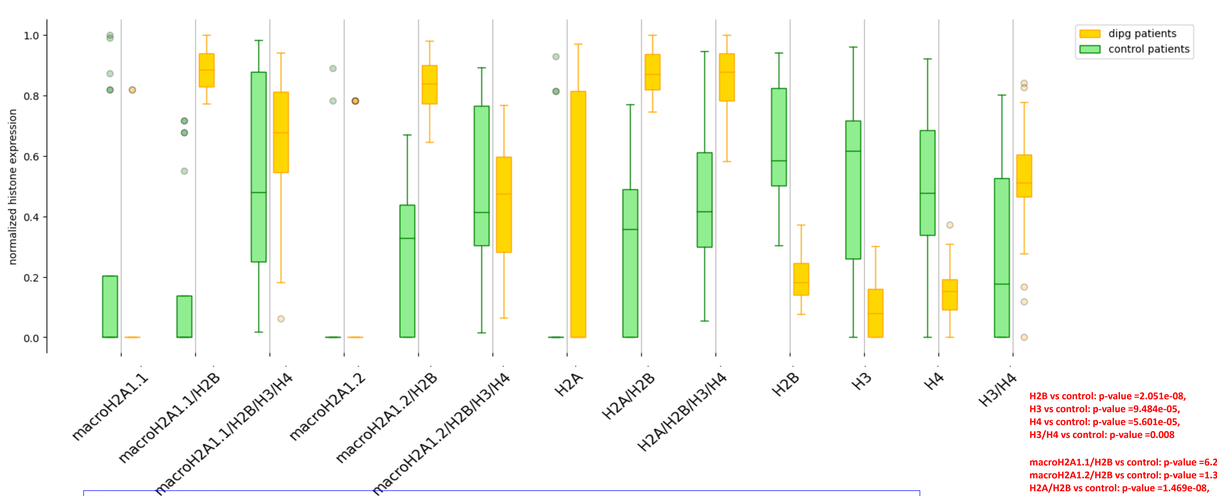


Figure 3. Individual histones are decreased, and histone complexes are increased, in the blood of children with DIPG

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PROSPERITAS VESTRA FINIS NOSTRA!

References:
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2. Buzova D.. Vinciguerra M. Clin Epigenetics. 2020 Aug 20;12(1):126.

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