Neuro-Oncology
22(3), 307–308, 2020 | doi:10.1093/neuonc/noaa003 | Advance Access date 7 January 2020

Modeling DIPG in the mouse brainstem

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See the article by Patel et al. in this issue, pp. 381–392.

Diffuse intrinsic pontine glioma (DIPG) is the leading cause of pediatric brain tumor death, with a median survival of under a year.[1] DIPGs are surgically inaccessible, radiotherapy is generally palliative, and standard chemotherapy and targeted agents are generally ineffective. The genomic era, driven first by array- and then next-generation sequencing–based analyses, has yielded much critical information on the mutations arising in DIPG, principal among them K27M substitutions in H3.3 (*H3F3A*) and H3.1 (*HIST1H3B*), which occur in around 80% of patients.[2] Other high-frequency mutations include platelet derived growth factor receptor A (*PDGFRA*) amplification or mutation (~40%) and *TP53* inactivation (~80%). DIPGs are genetically highly distinct from adult glioblastomas, which typically do not have K27M mutations, and although they also have a high frequency of Ras/mitogen activated protein kinase (MAPK) pathway alterations, most commonly these involve epidermal growth factor receptor, particularly the variant III rearrangement (EGFRvIII).

Given that many historical attempts to treat DIPG have stemmed from efforts in adults, the mutation landscape of DIPG perhaps partly reflects the still dismal prognosis for patients with this devastating disease. Furthermore, it is only in the last few years that studies have been able to attempt to generate preclinical models that accurately reflect the genetic and/or phenotypic background of DIPG. In vivo studies have largely focused on combining H3.3K27M with p53 and/or Ras/MAPK activation (via overexpression or mutation of *PDGFRA*, or *PDGFB* overexpression). These have included retroviral delivery into neonatal mice (RCAS [*replication-competent avian* sarcoma-leukosis *virus* long terminal repeat splice acceptor]), cortical in utero electroporation (IUE), and creation of a transgenic mouse.[3–5] They have all demonstrated a synergistic role for H3.3K27M in driving tumorigenesis together with p53/MAPK alterations.

In this issue, Patel et al[6] describe a brainstem IUE model combining H3.3K27M and dominant negative p53 (DNp53) with expression of either *Pdgfb* (a common tool used to generate murine glioma, although it is rarely amplified in DIPG), $Pdgfra^{WT}$ (a model of *PDGFRA* amplification), or a constitutively active $Pdgfra^{D842V}$. IUE was performed into the fourth ventricle at E13.5 with green fluorescent protein–positive cells migrating throughout the brainstem by postnatal day p21. These cells developed into oligodendrocytes, astrocytes, and neurons, indicating that progenitor populations were successfully targeted. Fully penetrant, invasive brainstem gliomas developed in electroporated mice with histopathological features of DIPG. PDGFB drove rapidly forming grade IV gliomas (glioblastoma), $PDGFRA^{D842V}$ mice developed grade III gliomas with more moderate latency and aggressiveness, and $PDGFRA^{WT}$ mice took over 200 days to develop gliomas, of which half were low grade (grades I/II). PDGFB tumors were extremely aggressive, with areas of vascular proliferation that the authors showed resulted from paracrine effects of PDGFB driving vascular remodeling in *Pdgfra* and *Pdgfrb* expressing cells, while $PDGFRA^{D842V}$ tumors had essentially normal vasculature.

H3.3K27M made no difference to the latency of PDGFB tumors, reflecting the aggressive nature of PDGFB/DNp53-driven murine glioma. However, H3.3K27M significantly accelerated tumor development in $PDGFRA^{D842V}$/DNp53 mice, perhaps because tumors took longer to develop due to more moderate Ras/MAPK activation. H3.3K27M induced large transcriptional changes in both PDGFB and $PDGFRA^{D842V}$ tumors, in agreement with previous RNA sequencing on fresh tumors[4] but in contrast to experiments where either samples were cultured prior to analysis or cell line experiments were performed.[5,7] Genes whose promoters are marked by H3K4me2 and H3K27me3 in progenitor cells, as well genes involved in glial identity and fate, were upregulated by H3.3K27M in both PDGFB and $PDGFRA^{D842V}$ tumors, while *Cdkn2a* (a major polycomb repressive complex 2 target that is downregulated in vitro by H3K27M and is important in mediating the effects of enhancer of zeste homolog 2 inhibition[8]) was downregulated.

PDGFB tumors were associated with a proneural gene signature, while PDGFRA^{D842V} tumors had enriched classical/mesenchymal signatures. Interestingly, when PDGFRA^{D842V} tumors were isolated and cultured, the tumor cells upregulated proneural genes and downregulated mesenchymal genes. This likely reflects differences in the tumor microenvironment of the PDGFB and PDGFRA^{D842V} tumors. Together with the transcriptional effects of H3.3K27M, these results highlight the major differences in findings that can be obtained from analysis of samples acquired directly from the in vivo model compared with in vitro culturing beforehand.

These IUE models permit reliable generation of spontaneous brain tumors that reflect the genetic and histopathological characteristics of human DIPG. Unlike xenografted cell lines, IUE targets in vivo cell populations in the context of a functional immune system. IUE and transgenic mice allow spatial control over oncogene expression as well as targeting of specific pre- and postnatal developmental windows; RCAS offers the same options to target postnatal populations. Transgenic mice allow more precise targeting of specific cell populations than IUE and RCAS, and a major limitation of IUE is the technical challenge of manipulating embryos in utero. Set against this, however, is the high penetrance and short time of tumor formation following IUE compared with transgenic mice, and thus all these methods have their relative advantages. As more preclinical DIPG models are developed, further information is gleaned about the spatial and temporal characteristics of tumor formation. For example, cortical expression of DNp53 and H3.3K27M generated tumors following IUE at E12.5,[4] while brainstem IUE of DNp53/H3.3K27M here at E13.5 did not. Future models carefully dissecting the spatiotemporal requirements for tumor formation will be required to fully understand the tumor cell of origin for DIPG and how that might vary with different genetic aberrations.

One major as yet unaddressed question is the direct role of H3.3K27M in driving tumorigenesis. Here, as in previous studies, H3.3K27M was able to accelerate tumors driven by other oncogenes. To date, using either in vivo or in vitro models, H3.3K27M alone has not been shown to itself directly induce tumor formation. This is in stark contrast to analysis of the clonal evolution of DIPG showing H3K27M to be the initiating mutation for DIPG,[9,10] and data showing that removal of H3.3K27M by CRISPR/Cas9 abrogates tumor formation in xenografted DIPG cells.[7]

Overall, the rapid development and full penetrance of tumors developed here make IUE a useful, albeit technically challenging, tool that, in conjunction with other types of mouse model, will allow improved in vivo testing by the community.

This text is the sole product of the authors and no third party had input or gave support to its writing.

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