Prdm8 regulates pMN progenitor specification for motor neuron and oligodendrocyte fates by modulating the Shh signaling response

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MS TITLE: Prdm8 regulates pMN progenitor specification for motor neuron and oligodendrocyte fates by modulating Shh signaling response

AUTHORS: Kayt Scott, Rebecca O'Rourke, Austin Gillen, and Bruce Appel

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. Both Referee 2 and 3 ask for more details and clearer evidence of the changes in Shh signalling and neural tube patterning in the Prdm8 mutants; I agree that this would strengthen your study. In addition, all three referees make several additional points that should help you revise the manuscript. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

Motor neurons (MN) and oligodendrocytes (OL) arise from the same progenitor domain in the ventral neural tube. Mechanisms underlying the switch in this progenitor domain from generating MN to OL are not completely understood. This study addresses the role of Prdm8 in the transition of motorneuron progenitors to OPCs in the ventral spinal cord of zebrafish. They first perform a characterization of the temporal and spatial characteristics of Prdm8 expression using scRNA-seq data from Olig2GFP sorted cells at 3 stages, followed up by FISH, and show pMN progenitors and OPCs express prdm8 and prdm8 expression declines as oligodendrocytes differentiate. They generated 2 CRISPR indel fish strains that are predicted to have a translational stop that would not translated the required ZF domains. A close look at the oligodendrocyte lineage shows premature switching from MN to OL lineage and premature differentiation from OPC to OL in homozygous mutants. This is done by IHC for Sox10 that marks the lineage and a series of transgenic fish that mark different stages of the OL lineage. Using BrdU and Isl1 they show there is a decrease in MN and BrdU+ cells at the different timepoints switch to the OL lineage. They also show that Patched is up in the mutants and with Shh inhibitor the MN phenotype can be rescued. The findings presented in the manuscript support the conclusions made and add an important player controlling the switch from generating MN to OL.

Comments for the author

1. Fig. 4 and Fig 8 have ectopic OL lineage cells dorsally in the Prdm8 mutants. In Fig. 8 legend it says these are dorsally migrated. Could they just be ectopic mis-specified cells similar to what was seen for Olig2 in the mouse Prdm13 mutants, Mona 2017? This ectopic expression could result from loss of a dorsally expressed Prdm8. Looks like in Fig. 1A at 24hpf there might be this expression. This is worth addressing in the discussion as an alternative explanation for the dorsal Olig2 cells.

2. The indels are predicted to be a truncation that should be loss of ZF domains. Whether this is acting as a null would be strengthened if the mRNA was gone in the mutants due to RNA missense mediated decay. Was there any attempt to use Prdm8 FISH probes on the mutant or RTqPCR in the mutants to see if RNA gone? I'm assuming there is no antibody.

3. Experiment shown in Fig 5 should show the number of Sox10+ only cells in the mutant and wildtype at each stage in the graphs.

4. I couldn't find if it was clear if adult homozygous mutants were grossly normal. This should be mentioned if it wasn't—and how does this compare with Prdm8 mutants in mice?

5. The authors state in the discussion that "Identification of genes misregulated in prdm8 mutant embryos combined with determination of genomic loci targeted by Prdm8 should help uncover the regulatory function of Prdm8 in pMN progenitor specification." This would have been a nice addition to increase the impact of the findings to the field. Even without the genomic targeting, performing the scRNAseq in the mutants, similar to what they did in the wt, might have been revealing for the genes misregulated. It will probably be argued it is beyond the scope of the current project but it might have been quite revealing.

Minor corrections:
Fig. 9B is missing a WT label.
Fig. 6C the Isl and the Olig2GFP panels are switched and mislabeled.

Reviewer 2

Advance summary and potential significance to field

Throughout the developing nervous system, most progenitor types first produce neurons before switching to the generation of glial cell types at later developmental stages. Scott et al.

characterize the expression of the transcription factor Prdm8 and elucidate its function in motor neuron and oligodendrocyte progenitors in the zebrafish spinal cord. Using in-situ hybridization and RNA sequencing approaches, they show that prdm8 is expressed in ventral progenitors, including motor neuron progenitors and OPCs, but not mature oligodendrocytes. To address the function of Prdm8 they generate 2 loss-of function alleles by CRISPR/Cas9 genome editing. In these mutants, the phase of motor neuron generation is shortened, OPC generation commences at an earlier developmental stage and a higher proportion of OPCs differentiate into oligodendrocytes. Lastly, they demonstrate that the earlier transition from motor neuron to OPC generation is due to elevated Shh signalling in ventral progenitors and that this phenotype can be partially rescued by inhibiting Shh pathway activity. Based on these data, they propose a model in which Prdm8 controls the timing of the switch from motor neuron to oligodendrocyte generation by repressing Shh signalling in pMN cells and the rate of OPC differentiation independently of this effect. This is a well-executed study that furthers our understanding of the genetic networks and molecular mechanisms that control the timing of motor neuron and oligodendrocyte development. The manuscript is well written and the logic of the paper easy to follow. The experiments are thoroughly quantified convincingly support the statements made by the authors and are clearly presented in the figures. I therefore believe this manuscript will be a valuable addition to the field.

Comments for the author

Major comments:

My only major comment is that I think a more detailed characterization of the prdm8 mutant phenotype would further improve this already really strong manuscript. The authors demonstrate that these mutants display increased Shh pathway activity in the ventral spinal cord at early stages of development, leading to the question if this causes an overall dorsal-ventral shift in gene expression boundaries or changes in the sizes of progenitor domains in the ventral spinal cord? The same group also demonstrated previously that motor neurons and oligodendrocytes arise from distinct cell lineages and that progenitors giving rise to oligodendrocytes typically initiate olig2 expression later than those giving rise to motor neurons in a process called “progenitor recruitment”. I am therefore wondering if the increase in Shh signaling in prdm8 mutants leads to an increase of early olig2-expressing cells acquiring a p3 identity which may be balanced by earlier onset of “progenitor recruitment” from dorsal domains, thus explaining the earlier depletion of actual motor neuron progenitors and earlier onset of OPC production.

Minor comments:

1) The authors demonstrate by BrdU incorporation that Sox10-positive oligodendrocytes are generated earlier in the pMN domain. Do the authors observe a similar earlier generation of glial cells from progenitors in p1 and p2 domains in which prdm8 is also expressed?

2) While the paper is generally well written and easy to follow, I am a little bit confused which aspect of oligodendrocyte differentiation Prdm8 precisely controls: the rate of differentiation of OPCs into myelinating oligodendrocytes or the developmental time at which OPC generation terminates or both? It would be great if the authors could clarify this aspect more during the revisions. Related to this, is it known if early myelinating oligodendrocytes and OPCs which persist into later stages are preferentially born at different timepoints?

Line 243: I think “possibly” should be “possibility”

Fig. 6C: The Isl and olig2:EGFP panels seem to be inverted.

Reviewer 3

Advance summary and potential significance to field

In the paper entitled “Prdm8 regulates pMN progenitor specification for motor neuron and oligodendrocyte" fates by modulating Shh signaling response”, Scott and colleagues investigate the

function of Prdm8, a histone Methyltransferase, during the fate switch of Olig2+ spinal cord progenitors from motor neurons (MN) generating cells to glial fated ones.

Prdm8 is a member of an enzyme family that recently merged as key component of gene networks controlling the emergence of patterned cellular diversity within the central and peripheral nervous system. A mouse mutant for Prdm8 has been created and revealed that its activity is required for the acquisition of specific terminal neuronal traits in the eye and brain (doi: 10.1073/pnas.1505870112; 10.1016/j.neuron.2011.09.035). Its functions during spinal neurogenesis remains unexplored.

Here the authors focus on the mechanisms controlling of the timing of neurogenic to glial switch of Olig2+ spinal progenitors in zebrafish; central to this switch is an timely controlled increase within the Olig2+ progenitors of Shh signalling. A very detailed expression profiling combining single cell RNAseq of Olig2 expressing cells and fluorescent in situ indicate that Prdm8 is present with Olig2+ progenitors during the switch. The careful phenotypic characterization of two newly generated null mutants for Prdm8 demonstrates that Prdm8 limits the production of Glial cells (Oligodendrocytes). This could be due to Prdm8 inhibition of Nkx2.2 expansion within Olig2+ cells, expansion known to be required to the glial switch of Olig2+ cells and/or to Prdm8 mediated inhibition of the differentiation of Olig2+ oligodendrocytes precursor cells (OPCs) into mature as Oligodendrocytes. Finally, a series of experiments suggest that part of these phenotypes are due to an interplay between Prdm8 and Shh signalling. Ptch2 a target of Shh signalling is increased upon loss of Prdm8. Decreasing Shh signalling with cyclopamine allows Prdm8 mutants to generate the appropriate number of MNs, however do not rescue the differentiation defects of OPCs into oligodendrocytes.

Overall the study is well conducted and bring new insights into the molecular tool kits controlling cell fate decision within the vertebrate embryonic spinal cord, yet the details means by which Prdm8 controls Olig2+ cell fate is not fully dissected, the interplay between Shh signalling and Prdm8 is unclear. It is worth mentioning that the scRNAseq data stands as a useful resource to many researchers in the field of spinal neurogenesis.

Comments for the author

-Prdm8 is expressed before and after the Switch of fate of Olig2+ cells, suggesting a timely regulated activity. Can the authors discuss this?
-The weak rescue of Prdm8 mutant phenotypes by cyclopamine argues that the modulation of Shh signalling by Prdm8 is only partially explaining Prdm8 activity during the fate determination of Olig2+ cells.
Do the authors can prove by other means that Shh signalling is elevated? Indeed the expansion of Nkx2.2 expression into the Olig2+ domains seen in Fig 8A is very mild.
Did the authors have investigated for the state of other usual suspects, such as Notch signalling?

Minor:
The labels and the figure legends needs to double checked, they contain several inversions (example: labels in Fig5C)

First revision

Author response to reviewers' comments

We greatly appreciate the reviewers' positive assessment of the quality and significance of our manuscript and their suggestions for improvement. Below we provide a point by point response to individual comments. We made several clarifications and corrections and we have been able to provide some new data to help strengthen our conclusions. All changes to the manuscript text are indicated by underlining.

Reviewer 1 Comments for the Author:
1. Fig. 4 and Fig 8 have ectopic OL lineage cells dorsally in the Prdm8 mutants. In Fig. 8 legend it says these are dorsally migrated. Could they just be ectopic mis-specified cells similar to what was seen for Olig2 in the mouse Prdm13 mutants, Mona 2017? This ectopic expression could result from loss of a dorsally expressed Prdm8. Looks like in Fig. 1A at 24hpf there might be this expression. This is worth addressing in the discussion as an alternative explanation for the dorsal Olig2 cells.

Response. This is an interesting possibility. However, we suspect that this is not the case. First, *prdm8* mutant larvae have no change in the total number of oligodendrocyte lineage cells. We would expect that an additional, ectopic source would increase the number of those cells. Second, there is no significant change in *olig2* expression in *prdm8* mutant embryos in contrast the ectopic dorsal expression of *Olig2* in *Prdm13* mutant mouse embryos reported by Mona et al. (2018). Finally, we do have some preliminary timelapse imaging data that do not reveal any evidence of dorsal oligodendrocyte lineage cell origins. We have not included those data in this manuscript because they are not sufficient to quantify and research limitations due to the COVID-19 crisis have prevented us from repeating that experiment.

2. The indels are predicted to be a truncation that should be loss of ZF domains. Whether this is acting as a null would be strengthened if the mRNA was gone in the mutants due to RNA missense mediated decay. Was there any attempt to use Prdm8 FISH probes on the mutant or RTqPCR in the mutants to see if RNA gone? I'm assuming there is no antibody.

Response. RT-PCR revealed that *prdm8* transcript levels are reduced, but not absent, in mutant embryos relative to wild-type embryos. We included these data as Figure 3E.

3. Experiment shown in Fig 5 should show the number of Sox10+ only cells in the mutant and wildtype at each stage in the graphs.

Response. We have now included these data as Figure 5E and Figure 5H.

4. I couldn't find if it was clear if adult homozygous mutants were grossly normal. This should be mentioned if it wasn't—and how does this compare with Prdm8 mutants in mice?

Response. We have been unable to recover homozygous mutant adults, which we now note in the Methods section. We have not performed a detailed analysis to identify the cause of lethality.

5. The authors state in the discussion that "Identification of genes misregulated in prdm8 mutant embryos combined with determination of genomic loci targeted by Prdm8 should help uncover the regulatory function of Prdm8 in pMN progenitor specification." This would have been a nice addition to increase the impact of the findings to the field. Even without the genomic targeting, performing the scRNAseq in the mutants, similar to what they did in the wt, might have been revealing for the genes misregulated. It will probably be argued it is beyond the scope of the current project, but it might have been quite revealing.

Response. We appreciate this insight and agree entirely with the point. Perhaps more important to us than the matter of scope is a matter of balancing impact with scientific training. As a graduate student, Kayt Scott, the first author, conducted almost all of the work described in this manuscript on her own. She also plans to conduct the follow-up RNA-seq and ATAC-Seq studies and to use those approaches to polish her bioinformatics skills. At the conclusion of her thesis project, she will have command of a broad and powerful range of experimental and analytical skills, which she might not attain if we were to pursue the team approach required for producing the comprehensive investigation suggested by the reviewer.

Minor corrections:
Fig. 9B is missing a WT label.
Fig. 6C the Isl and the Olig2GFP panels are switched and mislabeled.

Response. We have made these corrections.

Reviewer 2 Comments for the Author:
Major comments:

My only major comment is that I think a more detailed characterization of the prdm8 mutant phenotype would further improve this already really strong manuscript. The authors demonstrate that these mutants display increased Shh pathway activity in the ventral spinal cord at early stages of development, leading to the question if this causes an overall dorsal- ventral shift in gene expression boundaries or changes in the sizes of progenitor domains in the ventral spinal cord? The same group also demonstrated previously that motor neurons and oligodendrocytes arise from distinct cell lineages and that progenitors giving rise to oligodendrocytes typically initiate olig2 expression later than those giving rise to motor neurons in a process called "progenitor recruitment". I am therefore wondering if the increase in Shh signaling in prdm8 mutants leads to an increase of early olig2-expressing cells acquiring a p3 identity which may be balanced by earlier onset of "progenitor recruitment" from dorsal domains, thus explaining the earlier depletion of actual motor neuron progenitors and earlier
onset of OPC production.

Response. This is a very good point. We have struggled to address it because we still lack the fate mapping tools and domain identity markers we need to accurately discriminate between the origins and fates of adjacent cells within living embryos of different genotypes. Although we realize that this does not directly address the question, we now provide additional evidence of a change to Shh-dependent dorsoventral patterning by showing that *prdm8* mutant embryos have lower expression levels of *boc*, which is negatively regulated by Shh, than wild-type embryos. These new data are shown as Figure 9H,I.

Minor comments:
1) The authors demonstrate by BrdU incorporation that Sox10-positive oligodendrocytes are generated earlier in the pMN domain. Do the authors observe a similar earlier generation of glial cells from progenitors in p1 and p2 domains in which prdm8 is also expressed?

Response. This is good question. However, radial glia are numerous and they occupy the entire dorsoventral extent of the spinal cord. Markers that might distinguish between subtypes of radial glia and "radial astrocytes" remain poorly defined for zebrafish. Consequently, we lack markers of other glial types that would permit us to investigate that question.

2) While the paper is generally well written and easy to follow, I am a little bit confused which aspect of oligodendrocyte differentiation Prdm8 precisely controls: the
rate of differentiation of OPCs into myelinating oligodendrocytes or the developmental time at which OPC generation terminates or both? It would be great if the authors could clarify this aspect more during the revisions. Related to this, is it known if early myelinating oligodendrocytes and OPCs which persist into later stages are preferentially born at different timepoints?

Response. The reviewer undersold the importance of this question by listing it under "Minor comments". In fact, we think that making this distinction is very important, but at the moment we just don't know the answer. We suspect that Prdm8 regulates both OPC formation from pMN progenitors and subsequent differentiation. We hope that the RNA-seq and ATAC-seq studies that we will initiate as soon as we emerge from the current limitations to research due to the COVID-19 crisis will give us some insights. Additionally, we need a way to conditionally eliminate Prdm8 from oligodendrocyte lineage cells. A recent paper from Dave Lyons' lab showed evidence of oligodendrocyte-specific mutagenesis using a targeted CRISPR/Cas9 approach in zebrafish. We will attempt to adopt a similar approach to further test Prdm8.

Line 243: I think "possibly" should be "possibility"

Fig. 6C: The Isl and olig2:EGFP panels seem to be inverted.

Response. We made these changes.

Reviewer 3 Comments for the Author:
-Prdm8 is expressed before and after the Switch of fate of Olig2+ cells, suggesting a timely regulated activity. Can the authors discuss this?

-The weak rescue of Prdm8 mutant phenotypes by cyclopamine argues that the modulation of Shh signalling by Prdm8 is only partially explaining Prdm8 activity during the fate determination of Olig2+ cells.

Response. At the moment it is difficult for us to discriminate between the possibilities of a Shh-independent function of Prdm8 and a technical limitation of the cyclopamine rescue experiment. Zebrafish neural development is very rapid and we have found that it is extremely sensitive to small changes in the timing and dose of pharmacological inhibitors. Consequently, an incomplete rescue might be a consequence of an imperfect experiment.

Do the authors can prove by other means that Shh signalling is elevated? Indeed the expansion of Nkx2.2 expression into the Olig2+ domains seen in Fig 8A is very mild.

Response. We added new images and quantification of *nkx2.2a* expression to Figure 8. Additionally, we now provide new evidence of a change to Shh-dependent dorsoventral patterning by showing that *prdm8* mutant embryos have lower expression levels of *boc*, which is negatively regulated by Shh, than wild-type embryos. These new data are shown as Figure 9H,I.

Did the authors have investigated for the state of other usual suspects, such as Notch signalling?

Response. We have not investigated Notch signaling in the context of Prdm8 function. Understanding the dynamics of Notch signaling still challenges us. However, we have been quite excited to gain some new insights to Notch signaling within the pMN domain by analyzing our single cell RNA-seq data. We are preparing a manuscript describing those data now and will use this information for future investigations of pMN fatespecification.

Minor:
The labels and the figure legends needs to double checked, they contain several inversions (example: labels in Fig5C)

Response. We have made these corrections.

Second decision letter

MS ID#: DEVELOP/2020/191023

MS TITLE: Prdm8 regulates pMN progenitor specification for motor neuron and oligodendrocyte fates by modulating Shh signaling response

AUTHORS: Kayt Scott, Rebecca O'Rourke, Austin Gillen, and Bruce Appel
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

Motor neurons (MN) and oligodendrocytes (OL) arise from the same progenitor domain in the ventral neural tube. Mechanisms underlying the switch in this progenitor domain from generating MN to OL

are not completely understood. This study addresses the role of Prdm8 in the transition of motorneuron progenitors to OPCs in the ventral spinal cord of zebrafish. The findings presented in the manuscript support the conclusions made and add an important player controlling the switch from generating MN to OL.

Comments for the author

This revision addresses reviewer concerns. I have no further comments.

Reviewer 2

Advance summary and potential significance to field

Scott et al. characterise the role of the TF Prdm8 in controlling the timing of the switch from motor neuron to oligodendrocyte generation in the developing spinal cord of the zebrafish. They show that pMN cells in prdm8 mutants precociously switch from motor neuron to oligodendrocyte generation. Furthermore, oligodendrocyte generation also seems to terminate earlier in these mutants. They furthermore provide evidence that Shh signalling is elevated in these mutants and that treatment of prdm8 mutants with Cyclopamine restores the number of motor neurons. Based on these data they propose that Prdm8 controls the timing of the switch from motor neuron to oligodendrocyte formation by repressing Shh signalling in the ventral spinal cord. While the authors did not really address my questions from the previous round of review, I think this is an interesting and well-executed study. Since the molecular mechanisms that mediate the transition from neuro- to gliogenesis throughout the nervous system are still incompletely understood, I believe this manuscript will be a valuable addition to the field.

Comments for the author

The authors provide new data in the form of in-situ hybridisations for boc1, a gene negatively regulated by Shh, in Fig. 9H,I to provide further evidence that prdm8 mutants have elevated Shh signalling. Consistent with this hypothesis, they see a reduction in boc1 mRNA expression. Interestingly, this effect seems to be largely non-cell autonomous and there is a strong reduction on boc1 expression in the dorsal part of the spinal cord, where prdm8 is not expressed, at least in the images shown in Fig. 9H. How do the authors explain this and does this also translate into a ventralisation of progenitor and neuronal identities in the dorsal spinal cord?

Reviewer 3

Advance summary and potential significance to field

This is a very well conducted study that brings new insight into the regulation of cell fate switches during embryonic spinal cord development. The authors have improved their manuscript and have replied to all the questions raised by the referees.

Comments for the author

We think that the paper is ready to be published.