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Peer Review

Review of: "CHD3 Regulates BMP Signalling Response During Cranial Neural Crest Cell Specification"

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Here the authors are investigating the mechanisms of cranial neural crest cell (CNCC) differentiation by CHD3. CHD3, a nucleosome remodeling and deacetylation (NuRD) complex subunit, is an important regulator of craniofacial development. CHD3 mutations are implicated in craniofacial diseases. Using human induced pluripotent cells (hiPSCs) and CRISPR-mediated heterozygous and homozygous CHD3 loss of function, the authors are investigating how CHD3 mutations result in chromatin and gene expression defects that can explain disease. They have found that heterozygous CHD3 loss is dispensable, but homozygous CHD3 loss has temporal activities. It is dispensable for pluripotency but required for CNCC specification and differentiation. During CNCC differentiation, homozygous CHD3 loss facilitates the formation of aberrant mesodermal cells. The authors conclude that CHD3 primes the BMP signaling response by binding to BMP-responsive enhancers in developing CNCCs, and its loss causes chromatin remodeling defects that contribute to BMP and Wnt signaling imbalance, ultimately facilitating mesodermal cells and inhibiting CNCC differentiation. While Wnt signaling could partially restore CNCC differentiation, BMP signaling could not restore CNCC differentiation, indicating the temporal nature of CHD activities.

My comments:

1. Why are the heterozygous CHD mutants not recapitulating the CNCC phenotype? The authors have discussed compensation from the wild type (WT). But it could be due to the limited and buffered nature of the 2D differentiation system. Would a 3D differentiation help? Or is the timeline of differentiation itself not sufficient to capture the heterozygous phenotype?
2. As the first paragraph of the discussion repeats the introduction to CHD3, the authors might want to remove this paragraph.

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3. CHD3 homozygous loss does not affect pluripotency, as observed by colony morphology, mRNA expression, and protein expression by immunofluorescence of pluripotency markers. Do pluripotent genes shut off in DHD3 KO cells as they exit pluripotency and proceed towards CNCC differentiation? Does CHD3 have a role in exiting the pluripotency state?
4. Page 9, 2nd paragraph: Pluripotency genes were upregulated in CNCC cells at D18: As the authors showed that there is no difference between WT and KO cells at D0, I am assuming that the pluripotency genes were never downregulated during differentiation, unlike in WT cells. In that case, CHD3 would be a crucial factor that helps cells exit pluripotency. Can the authors comment on this?
5. *Fig 3c: While mesoderm development or differentiation is not enriched in the pathway enrichment, similarly, BMP signaling response is also not enriched in the pathway analysis. Can the authors perform GO Biological Process enrichment and present the data with statistics? Fig 3 Panels C-E feel like pick and choose and not using the global RNAseq analysis.*

Declarations

Potential competing interests: No potential competing interests to declare.