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Peer Review

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

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This *in vitro* study uses iPSCs to unravel the role of the NuRD chromatin remodelling complex member CHD3 for the induction of neural crest cells. They identify a major impact of CHD3 on the induction of early NC specification events such as EMT.

It appears that BMP/TGFbeta signaling is affected in mutant cells, with the ability to undergo EMT (a critical step for NCC delamination) being severely compromised. The authors suggest that this equates to an inability to induce a NC program; however, subsequent data very elegantly demonstrates that the loss of CHD3 leads to a change in the accessibility of sites for DLX5, which is associated with BMP signaling, and the predominance of Wnt signaling, which is also used in the differentiation protocol and drives mesoderm induction in the absence of BMP signaling.

This is a very interesting study illuminating the complex requirement for epigenomic regulation of signaling pathway effects to direct cell differentiation.

The interpretation of results is occasionally superficial or overenthusiastic and occasionally imprecise (see comments below). As a result, the outcomes of the study are somewhat difficult to follow up to the final experiments that illustrate the correlation between CHD3, BMP, and WNT signaling.

It is suggested to rephrase the interpretation precisely along these lines, as in vitro cell differentiation does not equate to what happens in vivo. These in vitro results show an inability to respond to BMP signaling, which is important for CNC specification in the protocol used. Compromised BMP signaling might be true beyond CNC induction.

Specific comments:

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Figure 1 establishes that undifferentiated iPSCs (both control and mutant) express pluripotency factors. No CHD3 protein expression was detected by Western blot in mutants; however, mRNA levels were only about 10-fold reduced in mutants. Care needs to be taken when calculating fold differential expression using qPCR, as background amplification of primer dimers or unspecific bands is often observed in cells deficient in a particular gene. Did the authors assess melting curves to ensure that the remaining expression is likely an artifact? Fig. S1 should show antibody staining in uninduced cells. As CHD3 is expressed in iPSCs, the RNAseq result of uninduced cells should be shown (and not only be mentioned).

Fig. S2: RNA-seq at d18 shows the differential expression of only a few genes deregulated in het mutants; however, these genes should be commented on. Similar to ko mutants, most genes belong to the Bmp or TGFbeta pathways (signaling or response), including genes involved in EMT (Smad 2, Snai2, Twist1, Zeb2, Vim) or maintaining epithelial structures (Bmp pathway). Note that the expression of these genes appears contradictory between Fig. 2A and Fig. 2C. The heatmap shows these genes in red, i.e., upregulated according to the legend provided (likely mislabelling, as red usually indicates downregulation). Interestingly, it appears that the top genes deregulated in mutant cells are already deregulated in het mutants. This should be commented on.

The description that CNCC-specific genes are being deregulated is not accurate. Most of the genes listed are not specific to CNCC, but to specific biological processes involved with CNCC.

Similarly, the statement ‘Together, these data suggest that CHD3 has an important role in CNCC specification’ is not correct. This data suggests that CHD3 plays critical roles in facilitating EMT required for CNCC specification. Fig. 2E requires quantification to support the statement on gradual upregulation (better to use the term ‘increase’, as the accumulation could also be due to increased protein stability). If RNAseq data is mentioned in the results from intermediate differentiation stages, this data needs to be shown.

The comment on compensation should be moved to the discussion.

Fig. 3 outlines ATACseq results showing major deregulation in chromatin accessibility. The authors suggest that most peaks fall into putative enhancers based on the criterion that they are > 1kb away from the nearest TSS. In this context, the range of distance needs to be explained. The term distal invokes a directionality. Please use another term.

CHD3 does not regulate genes. It appears to regulate chromatin accessibility through participation in the NuRD complex.

The interpretation that CHD3 fails to activate a CNCC program and activates mesoderm specification is only partially correct. The differentiation protocol used to obtain CNCC (BMPs) does not allow CNCC differentiation in mutant cells because this critical signaling pathway is interrupted. However, the authors did not test alternative protocols to ensure that CNCC induction proper is blocked.

Finally, in Fig. S3, justify the amount of rBMP2 used. Include a positive control showing that BMP2 is active (e.g., Smad6, Smad7), because the amount used is 10-100 fold lower than what is commonly used in vitro.

Declarations

Potential competing interests: No potential competing interests to declare.