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Influence of TADs boundary disruption on 3D genome organisation and genes expression at the mouse *Pdgfra/Kit/Kdr* locus

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Motivation and Aim: It is generally accepted that topologically associated domains (TADs) participate in genes expression regulation by bringing enhancer and promoter into proximity and maintaing their interaction. Whether TADs restrict ectopic enhancer interactions, thus exhibiting an insulation function, remains unclear. To explore the question we studied the role of 3D genome organization in the regulation of the *Pdgfra/Kit/Kdr* locus. The locus encodes tyrosine-kinase receptors, which are involved in cell differentiation processes. The locus consists of three TADs, so each gene is located within a corresponding TAD. Of note, the genes possess a distinct contrasting tissue-specific expression pattern: *Pdgfra* — fibroblasts; *Kit* — melanocytes, mast cells; *Kdr* — endotheliocytes (*Kdr* is also expressed in melanocytes, albeit weakly). We hypothesized that removal of TADs boundaries would provide ectopic enhancer interactions leading to aberrant genes expression in an unfamiliar cell type.

Methods and Algorithms: The mouse strains, carrying CRISPR/Cas9-mediated deletions of the key boundary CTCF-binding sites, and C57BL/6 mice as a control were used to obtain cell cultures (fibroblasts, mast cells, melanocytes). In order to analyse 3D genome organisation of the locus in the cell cultures we performed capture Hi-C and CTCF ChIP-seq, as well as H3K27Ac ChIP-seq to distinguish the enhancer pattern. To analyse a possible transcriptional change we obtained RNA from the cell cultures and proceeded with RNA-seq.

Results: For the boundary encompassing *Pdgfra* and *Kit* TADs, the deletion was not enough to disrupt the TADs insulation, as well as to activate *Kit* expression in fibroblasts. For the boundary encompassing *Kit* and *Kdr* TADs, the deletion revealed a tissue-specific influence. In mast cells the deletion led to a gain of inter-TADs contacts, but the *Kit* remained insulated from *Kdr* and no *Kdr* activation was detected. Conversely, in melanocytes the deletion did lead to TADs fusion and, most interestingly, to a noticeable activation of *Kdr* expression. There are at least two factors for such a difference. Firstly, unlike the mast cells, the melanocytes do express *Kdr* slightly. Secondly, in the mast cells *Kit* enhancers locate upstream of the *Kit*, while in the melanocytes — downstream, closer to *Kdr*. We presume that an epigenetic status of the locus and an enhancer location might contribute to TADs fusion restriction. In addition, the *Kit/Kdr* mutant mice demonstrated a phenotypic change, mutant mice being brown compared to the black control mice. The change putatively implies an extent of the *Kit/Kdr* TADs boundary evolutionary conservatism.

Conclusion: Considering these findings, we suggest that the TADs boundaries deletions are insufficient to change the regulatory pattern, unless the TADs fusion is allowed by other factors at least in the mouse *Pdgfra/Kit/Kdr* locus. Thus, the result provides an insight in a more complex TADs role requiring a comprehensive investigation of the factors participating in TADs formation.
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