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The role of the spatial chromatin structure in the regulation of the human keratin gene locus expression

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Motivation and Aim: The most notable example of the 3D genome organization influence on transcription is the gene expression switching in multigenic tissue-specific gene clusters. One of them is the keratin gene cluster which is located in chromosome 12 (12q13.13) and contains 26 genes. Potential locus control regions (LCRs) that we have previously discovered could be “switchers” of gene expression and transcriptional regulators of entire multigenic locus. Thus, the aim was to study the spatial structure of the keratin gene locus in mature keratinocytes as well as during the differentiation, including the analysis of LCR contribution in the formation of the locus topology. We also analyzed the mechanisms involved in the formation of the spatial configuration using drug treatments.

Methods and Algorithms: Immortalized normal skin keratinocyte cell line HaCaT, primary skin keratinocytes and iPSC-based epidermal differentiation model were used to analyze keratin gene locus structure. We performed 3C-based chromatin target enrichment method (C-TALE) and FISH coupled with confocal microscopy to obtain data about spatial configuration of the keratin gene locus. ChIP-seq method was used to analyze transcription factors and histone modification profiles in cell lines. We have also treated cells with a panel of small inhibitors to distinguish the effect of transcription and phase separation on the locus structure.

Results: We analyzed spatial chromatin structure of the keratin gene locus during differentiation of induced pluripotent stem cells (iPSC) using C-TALE. We found sequential formation of contacts between LCRs and *KRT5* gene. At the intermediate stage of differentiation contact between LCR1 and LCR2 is established before *KRT5* gene activation. Gene activation is observed on the final day of differentiation accompanied with broad H3 lysine 27 acetylation of *KRT5* promoter as well as LCRs. CTCF and cohesin binding patterns, obtained using ChIP-seq, are present only in LCRs and absent in *KRT5* promoter suggesting that different mechanisms contribute to the contacts formation. FISH method confirms the data obtained by C-protocol and demonstrates the condensation of the keratin gene locus during differentiation. The formation of the transcriptional hub is also indicated by the partial colocalization of LCRs and *KRT5* promoter observed in mature keratinocytes (HaCaT cells) and differentiating cells. We also analyzed the role of transcription and phase separation in formation of specific locus topology in HaCaT and primary skin keratinocytes using drug treatment. Treatment with flavopiridol, inhibiting the transcription initiation of

RNA polymerase II, led to enhancing of existing contacts between *KRT5* and LCRs, but not between LCRs and to the formation of new contacts in gene locus. Conversely treatment with, triptolide, which leads to proteasome-dependent degradation of RNA polymerase II, causes a significant weakening of similar contacts. Treatment with inhibitors of the phase condensate formation – JQ1 and 1,6-hexanediol – shows similar effects as a triptolide, while the contact between LCRs still remains stable.

Conclusion: We suggest that LCRs may play an important role in spatial structure and transcription control within the keratin gene locus during differentiation. Cell treatments with inhibitors of transcription and phase separation, as well as ChIP-seq data indicate the presence of different mechanisms affecting the formation of regulatory interactions in chromatin locus. Contact between LCRs probably forms by cohesion-driven loop extrusion and does not depend on transcriptional status of the locus. Contrariwise KRT5 gene promoter is not bound by cohesin and CTCF, and its contacts are formed by the formation of phase condensates including activator transcription factors and RNA polymerase II.

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