DOI 10.18699/SBB-2022-060

Chromosome structural variations of the chicken erythroblast HD3 cell line identified by Hi-C

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Key words: chicken cell lines, chromosome structural variations, HD3, Hi-C, karyotype abnormalities

Motivation and Aim: Karyotype abnormalities are frequent in immortalized continuous cell lines, derived from either primary tumors or *in vitro* transformed cells. Structural variations in chromosomes (SVs) can cause dramatic changes in gene expression patterns through direct gene fusion or gene breakage or through disturbance of the spatial organization of gene regulatory framework. This, in turn, can affect cellular phenotype and behavior. While for many continuous mammalian cell lines chromosome SVs are systematically studied and documented, cell lines from other vertebrate models often lack such information. The chicken LSCC-HD3 cell line (HD3), generated from erythroid precursors by transformation with avian erythroblastosis virus (AEV), has been used as a chicken model for erythroid differentiation and AEV-induced hematologic malignancies since 1982 [1]. However, karyotype abnormalities in HD3 cell line have not been assessed. In the present study we analyzed genome spatial organization of HD3 cells by high throughput chromosome conformation capture (Hi-C), which proved to be an effective tool for delineating chromosome rearrangements in mammalian cells.

Methods and Algorithms: To detect large-scale chromosome SVs, we obtained Hi-C heatmaps of genomic interactions for HD3 cells and looked for aberrant patterns of high density interchromosomal contacts, which are the hallmarks of interchromosomal translocations. Intrachromosomal inversions, deletions and duplications were revealed by analyzing intrachromosomal contacts in HD3 Hi-C heatmap compared to Hi-C map of chicken embryonic fibroblasts with normal female karyotype (ZW), obtained earlier [2]. We also compared the profiles of A/B compartments and topologically-associating domains (TADs) between the two chicken cell lines. To verify our data, we utilized the computational algorithm HiCtrans [3] for identification of translocations and chromosome breakpoints, as well as FISH with probes to rearranged regions.

Results: By visual inspection of Hi-C heatmaps of HD3 cells we identified more than 25 interchromosomal translocations, involving regions of ≥ 200 Kb on both micro- and macrochromosomes, which were confirmed by HiCtrans algorithm. Interestingly, HiCtrans tool revealed dozens of additional smaller scale translocations, which were hard to detect by visual analysis of Hi-C maps alone. Based on the Hi-C heatmap pattern we classified most translocations as unbalanced, leading to the formation of heteromorphic chromosomes. In many cases of microchromosome rearrangements, a whole microchromosome participated in the emergence of derivate chromosome with other macro- and microchromosomes, resembling “chromosomal fusions” or Robertsonian translocations between acrocentric microchromosomes. Several *de novo* identified simple and complex chromosomal rearrangements, such as between GGA2

and GGA1qter, between GGA5, GGA4p and GGA7p, between GAA4q, GGA6 and GGA19, were confirmed by FISH with BAC-probes, GGA4p-q paints, centromere-specific probes and probes to different types of chicken tandem repeats. By using paint probe to sex chromosomes, we also validated the GGAW duplication in HD3 cells, which thus have a ZWW karyotype.

Conclusion: The HD3 chicken cell line has a severely rearranged karyotype with most of the chromosomes engaged in translocations. Hi-C allows to simultaneously assess the spatial genome organization and chromosomal aberrations in the karyotype of birds with a large number of microchromosomes, which can hamper the investigation of karyotype abnormalities by classical cytogenetic approaches. Our Hi-C data of genomic interactions for the HD3 cell line can be used for comparative studies, analysis of affected genes at breakpoint regions, and general mechanisms leading to karyotype instability.

Acknowledgements: The study is supported by the Russian Science Foundation (grant No. 19-74-20075) and was performed using the equipment of Resource Center "Molecular and Cell Technologies" (Research Park of Saint-Petersburg State University) and Skoltech Genomics Core Facility.

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