A specific chromatin structure and increased *RUNX3* expression contribute to the Hodgkin/Reed-Sternberg cell-specific phenotype

T. Jox[1, 2], T. Zimmermann[4], M. Bartkuhn[3], A. Bräuninger[2], S. Gattenlöhner[2], D. Körholz[1]

[1] *Uni-Klinikum Gießen Marburg, Pedeatric Hematology and Onkology, Gießen, Hesse, Germany*
[2] *Uni-Klinikum Gießen Marburg, Pathology, Gießen, Hesse, Germany*
[3] *Justus-Liebig University, Genetics, Gießen, Hesse, Germany*
[4] *Justus-Liebig University, Computational Bioligy, Gießßen, Hesse, Germany*

Introduction

Hodgkin Lymphoma (HL) is one of the most common lymphomas in the western world and frequent in children, teenagers and young adults. Hodgkin Reed/Sternberg (HRS) cells derive from germinal center (GC) B-cells with clonal immunoglobulin rearrangements which often carry function impairing crippling mutations that should cause apoptosis of GC B cells. HRS-cells, however, escape apoptosis. Furthermore, they exhibit nearly no expression of typical B-cell markers. It is assumed that constitutive signaling (JAK-STAT, NFkB, PI3K-AKT), changes in the microenvironment and Epstein Barr Virus infection contribute to the HRS-cell survival. Another possibility could be the loss of the normal B-cell phenotype. The causes for the encompassing loss of the B cell phenotype of HRS cells are so far largely unclear.
We hypothesized that changes of epigenetic features, which is a poorly explored field of HL research, could contribute to the loss of the B-cell phenotype.

Methods

We characterized the chromatin landscape of HSR-cell lines in comparison to various non-Hodgkin lymphoma (NHL) cell lines by ATAC-Seq. RNA-Seq was performed to verify the Data. Computing analyses of the data allowed the identification of target factors.

Results

We identified a HRS-cell specific chromatin structure. The pattern of differentially expressed RNAs was largely in concordance with changes in the chromatin structure, especially regarding the loss of the B-cell identity. Furthermore, we identified motives of DNA binding factors enriched in the open chromatin of HRS-cells as compared to NHL cells. The binding sites of these factors were mostly in intronic and intergenic regions of the genome, including cis-regulatory sequences like enhancer and isolators. One of the DNA binding factors which enriched binding sites in open chromatin is RUNX3 which binds to promotors and enhancers and also takes part in normal B-cell development in interplay with RUNX1. RUNX3 is strongly expressed in HRS-cells compared to NHL-cells and RUNX3 knock down caused a partial restoration of B-cell specific gene expression in HRS-cell lines.

Conclusion

The Results indicates that the high RUNX3 expression could contribute to the HRS-cell specific chromatin landscape and the loss of the B-cell phenotype.

Affix

References

[1] Küppers, Ralf, Andreas Engert, and Martin-Leo Hansmann. 2012. “Hodgkin Lymphoma.” *The Journal of Clinical Investigation* 122 (10): 3439–3447.

[2] Schwering, Ines, Andreas Bra, Ulf Klein, Berit Jungnickel, Marianne Tinguely, Volker Diehl, Martin-leo Hansmann, Riccardo Dalla-favera, Klaus Rajewsky, and Ralf Ku. 2003. “Loss of the B-Lineage – Specific Gene Expression Program in Hodgkin and Reed-Sternberg Cells of Hodgkin Lymphoma.” *Blood* 101 (4): 1505–12.

[3] Voon, D., Hor Y. and Ito Y. 2015. “The RUNX Complex : Reaching beyond Haematopoiesis into Immunity.” *Immunology* 146: 523–36.

[4] Lee, Jung-won, Da-mi Kim, Ju-won Jang, Tae-geun Park, Soo-hyun Song, You-soub Lee, Xin-zi Chi, et al. 2019. “RUNX3 Regulates Cell Cycle-Dependent Chromatin Dynamics by Functioning as a Pioneer Factor of the Restriction-Point.” *Nature Communications*.

[5] Wang, Dapeng, Huitian Diao, Adam J Getzler, Shane Crotty, Ananda W Goldrath, and Matthew E Pipkin. 2018. “The Transcription Factor Runx3 Establishes Chromatin Accessibility of Cis - Regulatory Landscapes That Drive Memory Cytotoxic T Lymphocyte Formation” *Immunity* 48 (4): 659-674.e6.