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Review PhD thesis Julia Paczkowska

I was asked by the Institute of Human Genetics of the Polish Academy of Sciences to review the PhD thesis of Julia Paczkowska. Her PhD thesis was performed in the group of Maciej Giefing, who supervised her projects. It is a pleasure for me to comply with this request.

Main topic of Julia´s thesis is the pathogenesis of classical Hodgkin lymphoma (cHL). As outlined in the introduction, cHL is one of the most frequent B-cell derived malignancies at least in the Western world. Intriguingly, cHL patients show a bimodal distribution with one peak encompassing young adults and another peak in elderly patients. Whereas treatment success among young patients is impressive (cHL is among the best curable malignant diseases), treatment success in elderly patients is limited. This also holds true in the era of CD30-targeting immunotherapy approaches and implementation of checkpoint inhibitors in cHL treatment protocols. Treatment success continously increases in young cHL patients, and reducing toxicity of treatment regimens is the main goal of most clinical trials including young HL patients. However, treatment success of elderly patients has not improved similarly, and prognosis at least of intermediate and advanced cHL stages of the disease is limited. Furthermore, apart from acute toxicity, current chemo- and radiotherapy protocols might result in late toxicities including severe organ damage. Thus, apart from the

medical need to improve treatment success for subsets of cHL patients, treatment-related toxicity should be reduced.

To persue these aims, Julia aimed to characterize disease-specific defects by the analysis of genetic and epigenetic alterations relevant to cHL biology. Ideally, therapeutic targets are not only specific for the respective tumor cells, but they also target (signaling) pathways essential for growth and survival of the cells. To develop such treatment approaches, the knowledge of the disease biology is mandatory, and Julia´s work follows this path. Julia´s thesis resulted in three publications in internationally recognized journals, which already points to the quality of her work. Thereby, two of the publications are original articles containing original research, whereas one publication reviews the current knowledge on microRNAs in cHL and draws, based on this analysis, conclusions for HL biology. The publications are as follows:

1. **Original Article**: **Paczkowska J** *et al.*, Expression of ELF1, a lympoid ETS domain-containing transcription factor, is recurrently lost in classical Hodgkin lymphoma. *Br J Haematol*, 2019; 185:79-88.
2. **Review**: **Paczkowska J** and Giefing M. MicroRNA signature in classical Hodgkin lymphoma. *Journal of Applied Genetics*, 2021 Feb 5. doi: 10.1007/s13353-021-00614-7. Online ahead of print.
3. **Original Article**: **Paczkowska J** *et al.*, The tumor suppressive mir-148a is epigenetically inactivated in classical Hodgkin lymphoma. *MDPI Cells*, 2020; 9:2292.

In general, the thesis of Julia contains two parts. In the first part (publication 1), the question of deregulated transcription factors (TFs) in cHL is addressed. The second part (publications 2 and 3) refers to the role of microRNAs in cHL. The projects required a broad methodical pattern, and thus Julia achieved broad technical insights and skills. Due to the fact that Julia prepared a cumulative thesis, not all methodical details are outlined and can be judged completely. However, throughout, the original research data presented in the manuscripts are of high quality, which argues for the skills and technical standard Julia has achieved during her time in the lab of Maciej Giefing.

As already mentioned, the first part of the thesis addresses the question of deregulated TFs and their role for cHL biology. In the introduction, the current knowledge of deregulated TFs in cHL is outlined. Apart from TFs involved in growth and survival of cHL tumor cells, Julia specifically focuses on TFs involved in the loss of B-cell identity of HL tumor cells. The malignant Hodgkin/Reed-Sternberg (HRS) cells originate from B cells, however these cells have lost more or less completely the B-cell-specific gene expression program. As described in the introduction, this loss of B-cell identity is a result of silenced B-cell TFs like E2A or EBF, primarily caused by functional inactivation and DNA methylation. Importantly, the loss of B-cell TFs has been linked to growth and survival of cHL tumor cells. Of particular interest for Julia´s first project are the previous observations that reconstitution of B-cell TFs was so far largely unable to restore the B-cell phenotype in the HRS cells, and that the ETS family TF *ETS1* is recurrently deleted in cHL. Therefore, it was the aim of the project to reveal other B-cell TFs affected in cHL, involved in the loss of B-cell phenotype as well as growh and survival.

Starting from the observation that HRS cells lack the TF ETS1, Julia investigated the ETS domain-containing TFs ELF1 and ELF2 in cHL. First, expression of both TFs was analyzed by immunohistochemistry in a series of cHL cases. These analyses revealed that ELF1 expression was reduced or even lacked in the HRS cells. However, ELF2 expression was rather up-regulated in the majority of cases. Subsequently, cHL cell lines were analyzed for ELF1 expression. These analyses revealed that ELF1 expression was virtually absent in HRS cell lines. To elucidate the mechanisms of the lost ELF1 expression in HRS cells, Julia searched for ELF1 mutations and performed methylation analyses of the ELF1 regulatory regions. Whereas no mutations were found, a unique hypermethylation of the ELF1 promoter region was identified in HRS cells. Finally, using FISH and FICTION, HRS cell lines and cHL primary cases were analyzed for deletions of the gene. These analyses revealed recurrent heterozygous deletions in cHL cell lines and primary biopsies.

Together, in her first project Julia provides a comprehensive description of lost ELF1 expression in cHL, and convincingly releaves the mechanisms leading to its lost expression. This work leads to further questions, which could be addressed in future studies. It could be discussed why only ELF1 and not ELF2 is recurrently lost in the HRS cells. In their discussion, the authors speculate that ELF2 might substitute for the loss of ELF1 and ETS1, how could this be addressed experimentally? Is there a correlation with Epstein-Barr-virus infection? Furthermore, it would be interesting to

me to speculate why ELF1 genomic deletions are heterozygous but not homozygous. Might there be selection pressure to maintain at least one functional ELF1 allele? Finally, which experiments could be performed to address the functional relevance of the lost ELF1 expression in HRS cells?

As a general remark on Julia´s thesis, I realized that the most recent cited literature is from 2018 (with only a few citations from 2018; most of the citations from preceeding years). According to Julia´s opinion, are there no relevant findings on the topics of the thesis for cHL biology beyond 2018?

In the second part of her thesis Julia addresses the role of microRNAs in cHL. To clarify the current knowledge on microRNAs in cHL, Julia summarized in her second publication studies published so far on the topic. In this article, not only the current available literature is summarized, but also the authors speculate on the functional role of the deregulated microRNAs. The authors describe, that in cHL deregulated microRNAs are involved in impaired B-cell development, NF-κB activation and immune evasion, processes that are all highly relevant for cHL biology. They also describe that some of the deregulated microRNAs are cHL-specific and thus could serve as biomarkers for the disease. They conclude from this analysis, that further research is required to get in-depth insights into the microRNA landscape in cHL, and how microRNAs are deregulated in cHL.

These topics are addressed in Julia´s third publication. First, as a result from small RNA-seq analyses of cHL cell lines in comparison to non-Hodgkin cell lines and nomal B-cells, more than twenty down-regulated microRNAs were identified in cHL cell lines. Subsequently, the question was addressed, whether the downreglation of selected microRNAs (those with promoter regions located within <1000 bp fom a CpG island) correlated with DNA hypermethylation. Four of the five selected microRNAs showed an inverse correlation between DNA methylation and their respective expression. Remarkably, because of their exclusive hypermethylation in cHL cell lines, miR148-3p and miR148-5p were not only analyzed in the cell lines but also by pyrosequencing of microdissected primary HRS cells, which confirmed the cell line data. Furthermore, the authors identified, by a bionformatic analysis, putative mir-148a targets, and analyzed their respective expression in cHL cell lines. Indeed, six predicted genes were overexpressed in cHL compared to non-Hodgkin cell lines. Finally, the effects of mir-148a expression in cHL cell lines was investigated. In one of three anlyzed cell lines (KM-H2), proliferation was reduced following mir-148a

expression. From this work the authors conclude that aberrant DNA methylation is involved in microRNA regulation in cHL, and that epigenetic inactivation of mir-148a might play an important role in cHL biology.

This work addressed an important issue of cHL, and there is a clear need to better understand the pattern and functional relevance of deregulated microRNAs in cHL. Julia´s work adds important informations here. In particular, the data sets can be used to identify further microRNAs specifically dereglated in cHL. There also remain questions to be answered, e.g. why there is only in one cell line a reduction of proliferation observed; are the expression levels of the bioinformatically predicted target genes altered following mir-148a expression; and, most importantly, it will be interesting to speculate at what step of cHL pathogenesis micro-RNA deregulation plays the most important role.

Together, Julia presents a fully convincing thesis, for which it is a pleasure for me to recommend her work to the scientific council of the Institute of Human Genetics (Polish Academy of Science) for the further steps of the PhD procedure. In addition, if available, I would fully support her thesis to be awarded, as the thesis qualifies in my view for the grade „summa cum laude".

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