Review of: "Epigenetic regulator genes direct lineage switching in MLL-AF4 leukaemia"

Katrin Ottersbach[1]

1 University of Edinburgh

Potential competing interests: The author(s) declared that no potential competing interests exist.

MLL-rearranged infant acute lymphoblastic leukaemia (ALL) carries a dismal prognosis with treatment outcomes having seen no significant improvement for decades. The advent of immunotherapies, such as CD19-directed CAR-T cell therapy, have brought promising results; however, there have been several cases where leukaemic cells have been able to evade therapy via a lineage switch which resulted in an often fatal relapse as acute myeloid leukaemia (AML). Very little is currently known about the molecular mechanisms underlying such a lineage switch, which is not helped by the rarity of such cases.

The study by Tirtakusuma et al. is, to my knowledge, the most comprehensive thus far and delivers a wealth of much needed insights into the process and potential drivers of lineage switching, using 10 matched pairs of MLL-AF4+ ALL prognosis and AML relapse samples from 6 infant, 2 paediatric and 2 adult patients and comparing these with 2 cases of mixed phenotype (B lymphoid/myeloid) acute leukaemia (MPAL). They first of all demonstrate that these 10 cases indeed represent true lineage switches as the clinical parameters identify the prognosis sample as ALL and the relapse sample as AML, yet they share identical MLL-AF4 breakpoints, ruling out that the AML relapse may be secondary leukaemia related to therapy.

They further investigate the potential cellular origin of the lineage switch through analysis of B cell receptor rearrangements in the ALL and AML blasts and identify three patterns: (1) no rearrangements at the BCR locus in AML blasts, (2) shared rearrangement patterns in ALL and AML blasts and (3) unique BCR rearrangement patterns in AML blasts. This implies a lineage switch potentially originating from uncommitted HSPCs (1), or from B cell committed precursor from the major clone (2) or from a subclone (3).

RNA sequencing was performed on 6 out of the 10 samples and confirmed a switch from a lymphoid to a myeloid transcriptional programme. Interestingly, a large proportion of direct MLL-AF4 targets was also differentially expressed, suggesting that a different subset of gene targets are activated by MLL-AF4 in different types of leukaemia. DNase hypersensitivity site (DHS) analysis on one patient demonstrates that the differential gene expression is driven by altered chromatin accessibility at transcription start sites and

enhancers enriched for lineage-specific transcription factor motifs. The authors also show evidence for alternative splicing as a contributing factor.

Most importantly, the authors perform exome sequencing in order to find mutations that might identify potential lineage switch drivers. This confirms the previously described largely silent mutational landscape in MLL-rearranged infant ALL with more mutations present in the relapsed AML cells, although this is highly variable and largely driven by 3 samples. There were also no obvious candidate drivers of lineage switching common to all samples. In an alternative approach, the authors concentrated on one patient sample and performed targeted sequencing in cells from different parts of the haematopoietic hierarchy in an attempt to identify the order of mutation acquisition. This pointed to mutations in the epigenetic regulators PHF3 and CHD4 as early events and thus potentially facilitating the lineage switch. In functional validation studies, they demonstrated that a knockdown of PHF3 and/or CHD4 initiated a myeloid transcriptional and surface phenotypic change in the MLL-AF4+ ALL cell line SEM at the expense of a lymphoid programme. There was further evidence from the mutational landscape and the RNA-Seq data that changes in components of the polycomb repressive complex 1 may also be involved in the lineage switch.

One limitation of the study is the small cohort of patients who, despite all having undergone a switch from MLL-AF4+ ALL to MLL-AF4+ AML, vary substantially in age and some clinical parameters such as time to relapse and outcome. This made it difficult to find underlying common mechanisms and did not allow correlating any of the findings with clinical parameters. Due to quality and abundance of available material, a large part of the analysis (xenotransplants in Fig. 2, t(4;11) detection in HSPC subsets in Fig. 3, DHS analysis in Fig. 5 and order of mutation analysis in Fig. 7C) was focussed on just one patient, LS01, who may actually be considered as a bit of an outlier as relapse occurred only after 4 years, whereas all other patients relapsed within 1 year. This patient also had a positive outcome, suggesting that he may have suffered from a less aggressive disease as there was also no CNS infiltration. Furthermore, xenotransplants of ALL blasts from this patient into MISTRG recipients (Fig. 2C) showed some CD33 positivity, which together with the fact that this patient displayed pattern 1, i.e. no BCR rearrangements in the AML blasts, suggests that the leukaemic cells may have had some intrinsic plasticity, possibly because the relapse was driven by the HSPC compartment. It would have been interesting to perform xenotransplants into MISTRG recipients and look for CD33 positivity with ALL patient samples that showed pattern 2 or 3 in their BCR rearrangements or with ALL patient samples that did not undergo lineage switching. Similarly, the detection of t(4;11) in different blood cell populations (Fig. 3A-C) should at some point be repeated with samples that have shown pattern 2 or 3 for their BCR rearrangements. It would also have been useful to include HSPC populations in the transcriptomic analysis since the comparison of ALL and AML blasts and the inclusion of the two MPAL samples would mostly highlight changes that have occurred as a consequence of lineage switching and not those that are causative.

This study is nevertheless an extremely relevant and comprehensive study that has revealed many of the mechanisms underlying and accompanying a lineage switch and which, through the exome sequencing data and the functional validation results, has highlighted potential regulators of lineage determination and lineage switching which represent an important starting point for future studies and for the analysis of additional patient samples. There is also evidence from the analysis of the mutational signature in the AML blasts that chemotherapy may have contributed to the lineage switch, which is an important finding.