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Commentary

Tracking chronic myelomonocytic leukaemia diversity at the single cell level

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Chronic myelomonocytic leukaemia (CMML) is a prototypic myeloid neoplasm that associates features of myelodysplastic syndromes and myeloproliferative neosplasms. This rare and severe overlap disease is a very diverse clinical entity. Yet, genomic underpinnings of CMML combine a restricted number of somatic mutations in DNA methylation, histone modifier, splicing factor and signaling genes [1]. The discordance between mutational simplicity and clinical heterogeneity has suggested multiple explanations, from underestimation of genetic diversity to a prominent role of epigenomic and cell extrinsic factors [2, 3].

In this issue of EBioMedicine, Wiseman et al. [4] analyze gene expression at the single cell level to track CMML diversity within and between individual patients. They focus on sorted bone marrow $Lin^{-}CD34^{+}CD38^{-}$ cells, a cell population enriched in disease initiating cells. This analysis, performed in seven patients compared to three healthy donors, identifies intra-patient heterogeneity at the stem cell level. It also suggests the persistence of healthy stem cells beside leukaemic cells, and shows differences in gene expression between CMML-1 and CMML-2, two disease categories defined by the percentage of blast cells in the bone marrow and a distinct outcome.

Considered together, CMML stem and progenitor cells with a $CD34^{+}CD38^{-}$ phenotype show the up-regulation of genes involved in cytokine and chemokine signaling pathways as compared to healthy donor cells. This up-regulation enforces the suspicion that the cytokine milieu, whose make-up involves immature and mature cells of the leukaemic clone as well as cells of the bone marrow stroma, plays a role in CMML initiation and progression [5, 6]. Differentially expressed genes also indicate an early engagement of CMML cells into granulo-monocytic lineage differentiation as compared to healthy donor cells.

Behind these common features, Wiseman et al. [4] observe that CMML samples demonstrate a striking inter-individual heterogeneity, each of the studied samples clustering separately on two-dimensional visualization of highly variable genes by the non-linear technique named t-Distributed Stochastic Neighbor Embedding (t-SNE algorithm). This diversity was confirmed by unsupervised iterative clustering of differentially expressed genes, which generated 17 clusters. While healthy donor cells almost exclusively matched with one of these clusters, variable fractions of CMML cells of each individual were assigned to several clusters, adding intra-clonal diversity to inter-individual heterogeneity.

Remarkably, all but one of the tested CMML samples contained a significant fraction of cells matching with the major healthy donor cell cluster, possibly indicating the persistence of wild-type cells, even though some differentially expressed genes could be detected in cells of this common cluster between patients and healthy donors. CMML cells that match with the main healthy donor cluster could be persistent wild-type cells modified by their pathological environment, or cells of the leukaemic clone with limited changes in gene expression.

The 2016 iteration of the WHO classification of myeloid malignancies outlined two parameters related to patient outcome. The fraction of bone marrow blast cells distinguishes CMML-0, CMML-1 and CMML-2, the latter category being defined by a fraction of blast cells between 10% and 20% of bone marrow cells and the worst outcome. Comparing single cell gene expression in CMML-0/1 and CMML-2, Wiseman et al. [4] identify striking segregation of CMML-2 $CD34^{+}CD38^{-}$cells, which is nicely illustrated by pseudotime ordering analysis. Interestingly, such a transcriptome-based dichotomy was not observed between dysplastic and proliferative CMML, the other distinctive categories identified by the WHO. This suggests that exacerbated sensitivity to cytokines and growth factors such as GM-CSF that drives the proliferative component of the disease [9] depends less on transcriptional changes in the stem and progenitor cell compartment.

The first analysis of gene expression performed at the single cell level in CMML identifies new components in disease diversity whose origin has now to be clarified. Previous analysis of CMML clonal architecture demonstrated the co-existence of stem and progenitor cells with various numbers of somatic mutations, contrasting with the exclusive expansion of the most mutated cells with differentiation [7]. This analysis also

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showed early clonal dominance, with inconstant detection of wild-type $CD34^{+}CD38^{-}CD90^{+}$ cells [7]. Integration of genotyping, eg, by using Genotyping of Transcriptomes (GoT) [8], and chromatin accessibility analysis, e.g., by using Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-Seq) [10], with single-cell RNA sequencing will further indicate the persistence of wild-type cells and to what extent their behavior is altered by a pathological environment. To be sustainably efficient, CMML therapy may promote the expansion of residual, wild-type stem cells and their differentiation into mature cells, which is hardly obtained with current therapies such as demethylating drugs [1]. A better understanding of disease diversity components may indicate the need to reprogram residual wild-type stem cells before expansion, guiding the choice of innovative strategies to develop in CMML.

Declaration of Interests

The author has no conflicts of interest to disclose.

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