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LEUKAEMIA

Powers of prediction

> a set of leukaemic cell features that could predict relapse at diagnosis

Relapse is thought to originate from resistant cancer cells that either pre-exist or emerge following drug treatment. If the former is true, identification and characterization of these rare cells through deep phenotypic single-cell analyses could have important implications for risk stratification. Testing this hypothesis at diagnosis, Good et al. investigated B cell precursor acute lymphoblastic leukaemia (BCP-ALL) cells at the single-cell level and found the presence of developmentally dependent cell signalling states correlating with relapse.

Initially, the profiles of primary diagnostic bone marrow (BM) samples from 60 patients with BCP-ALL were compared with BM aspirates from five healthy individuals using single-cell mass cytometry. As expected, this revealed a skewing of BCP-ALL cells towards early stages of B lymphopoiesis. Next, the authors generated a single-cell developmental classifier whereby healthy BM was categorized into 12 developmental populations defined by the expression of 11 proteins associated with B cell development. This served as a 'barcode' of phenotypic maturity, enabling each individual leukaemic cell to be matched to its closest developmental stage.

Classification of the BCP-ALL cells in this manner demonstrated that there was a significant expansion of cells across the pre-pro-B to pre-BI transition. Furthermore, the expanded leukaemic cell populations maintained the expression of 24 proteins involved in normal B cell development. Interestingly, the authors also found that single-cell developmental classification of leukaemic cells only weakly correlated with chromosomal rearrangements, which are often used to determine prognosis and risk.

Given that cells from patients who went on to relapse were not found to be enriched for a specific developmental state, the authors wanted to identify a set of leukaemic cell features that could predict relapse at diagnosis. Using a machine-learning approach called elastic net, Good et al. generated a relapse prediction model that they named developmentally dependent predictor of relapse (DDPR); this model identified six predictive features that could separate patients with respect to their relapse status. Specifically, these features were limited to the pro-BII and pre-BI cell populations and were linked to high basal activation of mTOR signalling in pro-BII cells and high basal activated and unresponsive pre-B cell receptor (BCR) signalling in pre-BI cells. Importantly, in evaluating the performance of the DDPR model, it was shown that its predictive power was most effective on diagnostic leukaemic samples that had been organized by single-cell developmental classification at the outset rather than on bulk unclassified cells. Moreover, combining the DDPR model with current risk-prediction methods improved patient stratification according to relapse-free survival on follow-up data at 5 years after diagnosis.

Lastly, taking into consideration that BCP-ALL cells are likely to adapt under therapeutic pressure, the authors examined seven paired diagnosis and relapse samples. Although the leukaemic cells had contracted almost entirely to the pre-BI population at relapse, the DDPR features associated with poor outcome at diagnosis persisted or became more prominent at relapse.

This study highlights how understanding the phenotypic heterogeneity of cancer by leveraging single-cell analyses can identify developmental signalling targets that could guide personalized treatment strategies.

Anna Dart

ORIGINAL ARTICLE Good, Z. et al. Single-cell developmental classification of B cell precursor acute lymphoblastic leukemia at diagnosis reveals predictors of relapse. *Nat. Med.* https://doi.org/10.1038/nm.4505 (2018)