Single-cell-based analyses emphasize 24h as a critical time-point for the commitment to the erythroid differentiation process

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Résumé

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Cell decision-making is the ability of any living cell to integrate variable environmental information into a coherent biological response. Our group is interested in understanding how such an integration is implemented during a differentiation process in metazoan cells. Although this issue has already been studied for many years, most analyses were based upon data acquired at the level of cell populations and only a response averaged over the population was considered. Yet it has become increasingly clear in the last few years that all cell populations present significant quantitative and qualitative heterogeneity that may be of utmost importance in living organisms. It can even be proposed that variability in gene expression could be a driving force in differentiation (Huang, 2010).
We decided to investigate this process at the single-cell level, to better understand the decision between self-renewal and differentiation, in T2ECs (Gandrillon et al., 1999). Those are primary normal chicken erythrocytic progenitor cells that can be maintained using appropriate factor combinations in a self-renewing state. By changing medium conditions those cells can be induced, at any time in culture, to terminally differentiate into fully mature erythrocytes.

We first performed a global population level approach using RNAseq, in order to compare a full list of gene expression levels between self-renewing and cells differentiated for 48 hours. Using a multicriteria methodology, we have then defined a set of the most relevant 96 genes that have then been investigated at the single cell level.

We therefore performed RTqPCR using the *BioMark*$^{\text{TM}}$ HD System on these 96 genes in 384 single-cells isolated at 0, 8, 24 and 72 hours of differentiation. At the population level, we showed that the different time-points could be clearly distinguished. In contrast, at the single-cell level, data were much more heterogeneous and complex. Indeed, cell individuality, hidden behind the averaging effect in populations was revealed at the single-cell level. We also computed a distribution-based correlation network per time-point that harbored time varying structures in term of density, nodes and hubs. The 24h network showed overall a different aspect with a much higher density of edges. We then calculated an entropy value to measure the variation in heterogeneity level. We observed that entropy increased during the differentiation process and reached a maximal value at 24h, before declining at 72h. Since correlation networks and entropy value emphasized the 24h time-point we made the hypothesis that this might be a crucial point in our differentiation process. Experimental data indeed confirmed those *in silico* predictions by demonstrating that the critical point of commitment in the differentiation process is located between 24h and 48h : cells induced to differentiate for 48h were not able to self-renew anymore, whereas cells induced for 24 h were clearly able to revert to a self-renewal state.

Huang, S. (2010). Cell Lineage Determination in State Space: A Systems View Brings Flexibility to Dogmatic Canonical Rules. PLOS Biol *8*, e1000380.
Gandrillon, O., Schmidt, U., Beug, H., and Samarut, J. (1999). TGF-beta cooperates with TGF-alpha to induce the self-renewal of normal erythrocytic progenitors: evidence for an autocrine mechanism. Embo J *18*, 2764-2781.