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ORAL ABSTRACTS

501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Characterization of Hematopoietic Stem Cell Diversity Using Spatiotemporal Quantitative Phase Imaging

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Cells undergo continual transformations, never retaining their original state. Hematopoietic stem cells (HSCs), at the apex of the hematopoietic hierarchy, are not exempt from this dynamic process. In recent years, the advent of single-cell analysis techniques such as scRNAseq has progressively unveiled the heterogeneity within cell populations previously presumed to be homogeneous. However, these techniques are limited to evaluating the cellular state at a single temporal point based on snapshot analysis, thus failing to account for temporal changes and dynamic behavior of cells. Therefore, the true diversity inherent to the ever-changing HSCs remains uncertain.

In this study, we comprehensively elucidate HSC diversity, including temporal changes, by integrating our recently established ex vivo HSC expansion techniques (Wilkinson et al., Nature, Becker et al., Cell Stem Cell), single-cell quantitative phase imaging (QPI), and post-transplantation dynamics analysis. For live imaging, HSCs were harvested from hepatic leukemia factor (*Hlf*)-tdTomato reporter mice, which specifically marks long-term HSCs. Index sorted single *Hlf*-tdTomato $^{+}$ HSC were expanded with a single-HSC culture system and monitored by QPI fluorescence time-lapse imaging. Surprisingly, even though the cells were derived from the same HSC fraction, significant variations in mitotic rate, motility, morphological changes, and *Hlf* expression levels were observed at the single-cell level. Stemness did not decrease stepwise over time, but always exhibited continuous changes. Thus, analysis including the temporal dimension illuminated the infinitely branched nature of HSC diversity.

Interestingly, *Hlf* expression level correlated with the dynamic behavior of HSCs in the expansion culture system. *Hlf*-high HSCs exhibited a slow division rate, small, rounded morphology and low motility, while *Hlf*-low HSCs actively divided and differentiated, displayed larger morphological size with low sphericity, and exhibited high motility. Furthermore, spatiotemporal dynamics analysis of *Hlf*-high/low HSCs after transplantation, employing the highly sensitive in vivo imaging system Akaluc bioluminescence imaging (AkaBLI), revealed that *Hlf*-high HSCs initiate systemic hematopoiesis slowly, while *Hlf*-low HSCs expanded quickly, characterized by short-term explosive hematopoiesis in the spleen. As expected, only *Hlf*-high HSCs had long-term bone marrow reconstitution ability. Thus, based on the expression level of *Hlf*, it is possible to predict the dynamic behavior of the cells in the expansion medium and in the post-transplant body.

Next, we investigated whether *Hlf* expression level could be predicted based on the kinetic features of cells during HSC ex vivo expansion. Interestingly, multidimensional analysis based on kinetic features extracted from time-lapse imaging showed multiple clusters, each linked to *Hlf* expression levels. In human HSCs, the dynamic diversity of HSCs was also observed in our recently established ex vivo expansion system (Sakurai et al., Nature). Notably, the kinetic features of human HSCs differed from those of mouse HSCs. Transplantation of human HSCs exhibiting specific morphological characteristics showed long-term reconstitution ability. Thus, classification of HSCs based on kinetic features facilitated enrichment of functional HSCs. This study demonstrates the importance of characterizing cells, including the temporal dimension, and illustrates the existence of infinite dynamic individuality in mouse and human HSCs.

Disclosures No relevant conflicts of interest to declare.

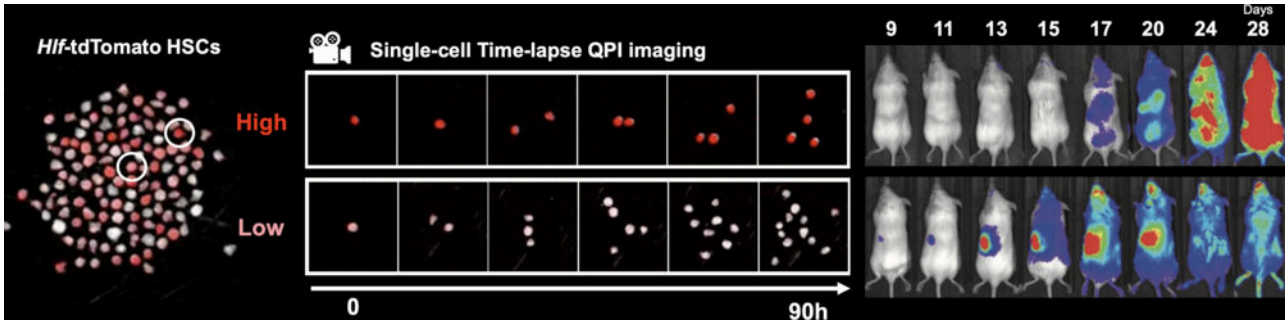

Figure 1

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