Single Cell Live Imaging Data Sheds Light on Megakaryocytic-Erythroid Progenitor

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To examine the decision-making process in progenitor cell differentiation, we developed a novel time-lapse microscopy approach to visualize single sorted bipotent Megakaryocytic-Erythroid progenitors (MEP), as well as unipotent Megakaryocytic and Erythroid progenitors as they proliferate and differentiate down their respective lineages. Utilizing image analysis, we traced the lineage history of committed cells from their parent cells and quantified features such as duration of cell cycle and movement characteristics at single cell resolution.

We propose that time-lapse microscopy technique and image analysis can shed light on significance of cell migration in cell differentiation process.

I. INTRODUCTION

BLOOD cancer is responsible for approximately 10% of all cancer cases in US every year [1]. Perturbation of normal hematopoietic differentiation leads to such cancers as leukemia, lymphoma and myeloma. Elucidating the mechanisms regulating hematopoietic stem and progenitor cell differentiation and fate decision is crucial for prevention and curing these diseases.

II. EXPERIMENT SETUP AND MODELLING

A. Cell Sorting and Dual Mk/E Colony Live Imaging Assay

Erythroid progenitors (ErPs) were sorted as $CD34^{+}Lin^{-}Flt3^{-}CD45Ra^{-}CD38^{high}Mpl^{-}$. Megakaryocyte progenitors (MkPs) were sorted as $CD34^{+}Lin^{-}Flt3^{-}CD45Ra^{-}CD38^{mid}Mpl^{+}CD41^{+}$. MEPs were sorted as $CD34^{+}Lin^{-}Flt3^{-}CD45Ra^{-}CD38^{mid}Mpl^{+}CD41^{-}$.

24 hours after plating, cells were imaged with an Olympus VivaView every 2 hours in DIC for the first 36 hours, and then 10 minutes in DIC for an additional 3.5 days. On day 7, antibodies were added to the dish, and imaging continued every 10 minutes in DIC, FITC, and Cy5 channels for an additional 24 hours. Images were processed and stacked with FIJI [2].

B. Tracking

Each stack of images was automatically tracked using the Baxter Algorithm developed by the Blau Lab at Stanford University [3] and manually corrected. Such quantifiable features of cells as cell cycle duration, coordinates and intensity of fluorescence were exported.

C. Data analysis

The cell coordinates were utilized to differentiate between the different types of movement: random walk or Levy flight, to calculate the velocities and distances travelled. Classification of cells was made based on this data.

D. Stochastic simulations

Gillespie algorithm was used to model MEP proliferation. To consider history dependent cell cycle, heterogeneity in MEP population was introduced.

III. CONCLUSION

We demonstrated that such features as cell cycle duration and motility characteristics permit predictions of probabilities of MEP lineage choices. Moreover, utilizing Gillespie simulations we were able to shed light on MEP biology and show the heterogeneity of this population. Our results serve as an illustrative application of single cell tracking technique of time-lapse imaged cells which are undergoing lineage commitment.

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