Molecular Aspects of Medicine xxx (2017) 1–10

Contents lists available at ScienceDirect

Molecular Aspects of Medicine

journal homepage: www.elsevier.com/locate/mam

Single cell analysis of normal and leukemic hematopoiesis

Benjamin J. Povinelli [a, b, 1], Alba Rodriguez-Meira [a, b, 1], Adam J. Mead [a, b, c, *]

[a] MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom
[b] Haematopoietic Stem Cell Biology Laboratory, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom
[c] NIHR Biomedical Research Centre, Churchill Hospital, Oxford, United Kingdom

ARTICLE INFO

Article history:
Received 22 May 2017
Received in revised form
17 August 2017
Accepted 28 August 2017
Available online xxx

Keywords:
Hematopoietic stem cell
Single cell RNA-seq
Hematopoiesis
Leukemia
Genomics
Heterogeneity

ABSTRACT

The hematopoietic system is well established as a paradigm for the study of cellular hierarchies, their disruption in disease and therapeutic use in regenerative medicine. Traditional approaches to study hematopoiesis involve purification of cell populations based on a small number of surface markers. However, such population-based analysis obscures underlying heterogeneity contained within any phenotypically defined cell population. This heterogeneity can only be resolved through single cell analysis. Recent advances in single cell techniques allow analysis of the genome, transcriptome, epigenome and proteome in single cells at an unprecedented scale. The application of these new single cell methods to investigate the hematopoietic system has led to paradigm shifts in our understanding of cellular heterogeneity in hematopoiesis and how this is disrupted in disease. In this review, we summarize how single cell techniques have been applied to the analysis of hematopoietic stem/progenitor cells in normal and malignant hematopoiesis, with a particular focus on recent advances in single-cell genomics, including how these might be utilized for clinical application.

1. Introduction

The hematopoietic system is perhaps the best-defined model of cellular differentiation due to ease of access, readily identifiable mature blood lineages and plethora of *in vitro* and *in vivo* assays. Hematopoiesis is organized as a hierarchical process originating from a rare population of multipotent and self-renewing hematopoietic stem cells (HSCs) that provide a life-long supply of multiple different types of morphologically distinct mature blood cells, through a series of intermediary progenitor cells. Consequently, the hematopoietic system is well established as a paradigm for the study of cellular hierarchies and their disruption in disease (Orkin and Zon, 2008; Ema et al., 2014).

The regenerative capacity of cells within the hematopoietic system was first demonstrated through the rescue of lethally irradiated mice by transplantation of untreated bone marrow (Till and McCulloch, 1961). Following these initial experiments, HSC transplantation in patients was established as a routine treatment, and this remains by far the most widely used regenerative therapy in medicine (Copelan, 2006). The occurrence of macroscopic spleen colonies in early transplantation experiments also suggested the high proliferative capacity of some single cells within the hematopoietic system and the consequent need for single cell assays to study normal hematopoietic function. Subsequent experiments using marrow from aneuploidy mice confirmed the unicellular origin of transplant-derived spleen colonies (Becker et al., 1963). Since these original observations, hematopoiesis has led the way in the development and application of a plethora of single cell phenotypic and functional analysis techniques to study blood cell development *in vitro* and *in vivo* (Fig. 1). It is perhaps not surprising, therefore, that hematopoiesis has also emerged as a key developmental system to apply recent technical advances in single cell genomics. According to Sydney Brenner, “Progress in science depends on new techniques, new discoveries and new ideas, probably in that order” (Brenner, 2002). As predicted, the application of new single cell methods to investigate the hematopoietic system has led to paradigm shifts in our understanding of cellular heterogeneity in hematopoiesis and how this is disrupted in disease. In this review, we summarize how single cell approaches have been applied to the analysis of hematopoietic stem/progenitor cells (HSPC) in normal and malignant hematopoiesis, with a particular focus on recent single-cell genomics techniques.

* Corresponding author. MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom.
E-mail address: adam.mead@imm.ox.ac.uk (A.J. Mead).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.mam.2017.08.006
0098-2997/

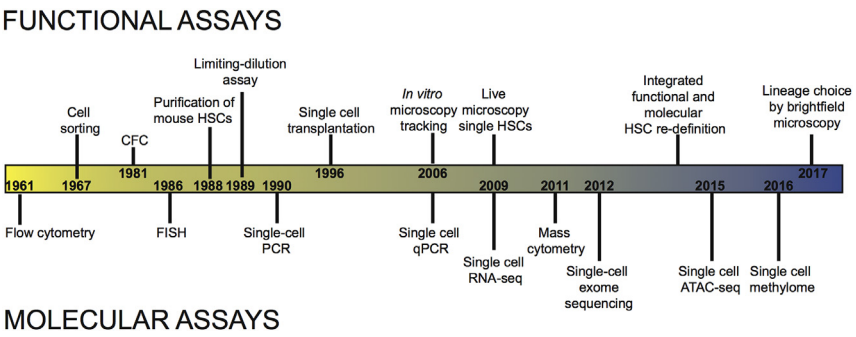

Fig. 1. Timeline illustrating key developments in the application of single-cell assays in hematopoiesis.

2. Single cell analysis and normal hematopoiesis

2.1. Limitations of phenotypically defined cell populations in hematopoiesis

The ability to prospectively isolate immunophenotypic subsets of bone marrow cells was established through the use of monoclonal fluorescent antibodies and fluorescence-activated cell sorting (FACS, Fig. 2A), pioneered by the Weissman laboratory. This single cell analysis method enabled the purification of a rare subset of bone marrow cells by excluding the cell surface markers for mature blood lineages (Lin-), and selecting for the cell surface markers Thy-1 and Sca-1 (Spangrude et al., 1988). The long term repopulating capacity of bone marrow was also shown to be confined to this subset (Uchida and Weissman, 1992). Subsequently, the phenotypic definition of HSCs has been further refined using a number of different markers, fluorescent dyes and/or transgenic mouse lines (Purton and Scadden, 2007). However, all methods to purify HSCs based on cell surface phenotype are limited by the same fundamental problem relating to heterogeneity within the phenotypically defined HSC compartment, including "contamination" by variable numbers of non-HSCs depending on the method used. Furthermore, purity of functional HSCs within the phenotypically-defined HSC compartment is affected, sometimes dramatically, by genetic background of mice, following perturbations such as 5-FU treatment and in disease models (Purton and Scadden, 2007). Heterogeneity within phenotypically defined stem/progenitor cell populations is particularly problematic in human hematopoiesis (Doulatov et al., 2012). Ultimately, any phenotypically defined hematopoietic cell population will encompass a range of heterogeneous cell-types. Assays of stem cell function and lineage potential at the cell population level obscure this heterogeneity and can lead to false conclusions, highlighting the need for single-cell approaches to study hematopoiesis (Fig. 2B–D).

2.2. Single-cell functional assays in hematopoiesis

Hematopoiesis has the considerable advantage of an array of *in vitro* and *in vivo* single-cell assays (Purton and Scadden, 2007; Ema et al., 2006). Early colony assays provided evidence of heterogeneity in self-renewal capacity and lineage potential of individual HSPCs (Humphries et al., 1981; Suda et al., 1983). Subsequently, methods were developed to purify and transplant phenotypically-defined single HSCs, yielding long term multilineage reconstitution (Osawa et al., 1996). This single cell transplantation approach has been used to characterize phenotypically defined HSCs, with ~15%–~67% of transplanted cells yielding long-term engraftment with mouse HSCs (Wilson et al., 2015) and up to ~20% with human HSCs (Notta et al., 2011). Importantly, single cell transplantation has also identified a striking degree of functional heterogeneity within the phenotypic HSC compartment with individual stem cells demonstrating mature blood cell output biases or restrictions (Dykstra et al., 2007; Sieburg et al., 2006; Yamamoto et al., 2013; Copley et al., 2012).

Similarly to the use of single cell *in vivo* experiments to resolve the phenotypic identity and functional heterogeneity of HSCs, single cell analysis has been instrumental in defining the major lineage branch points in the hematopoietic hierarchy. In the classical model of hematopoiesis, long-term HSCs differentiate through a series of increasingly lineage restricted progenitors concordantly with a loss of self-renewal (Babovic and Eaves, 2014). By sorting single cells for *in vitro* culture and identifying their lineage output, the pathways of progenitor differentiation have been identified (Fig. 3). As an illustrative example, in mouse hematopoiesis, this single cell analysis approach was used to revise classical models of hematopoietic differentiation through identification of the earliest lineage restriction step as a loss of megakaryocyte and erythroid potential in single cells sustaining other blood lineage developmental potentials; the so called lymphoid primed multipotent progenitor (LMPP) (Adolfsson et al., 2005). Lineage outputs from individual LMPPs *in vivo* have been shown to be more heterogeneous using cellular-barcoding approaches (Naik et al., 2013), however the sensitivity to detect lineage outputs of a particular clone *in vivo* using this approach remains to be established. Similarly, single cell *in vitro* studies have challenged classical models of human adult hematopoietic differentiation, raising the possibility of a two-tier hierarchy of multipotent and unipotent cells, without lineage restricted multipotent intermediaries (Notta et al., 2016). Together, these observations from single cell *in vivo* and *in vitro* functional assays have provide evidence of considerable functional heterogeneity even within the most stringently defined HSPC populations. Ultimately, the self-renewal and lineage potentials of cells residing within a HSPC population can only be definitively established through single cell analysis, including both *in vitro* and *in vivo* functional assays, and hematopoiesis has led the way in the development of such single cell approaches (Fig. 2D).

2.3. Single cell gene expression analysis applied to hematopoiesis

In parallel with single cell functional assays, early single cell gene expression analysis of HSPC provided evidence of transcriptional lineage-priming, preceding lineage specification (Hu et al., 1997). This method, which allows analysis of combinatorial gene expression in single cells, is particularly informative when integrated with functional single cell analysis. For example, this approach provided evidence for hierarchical organization of transcriptional lineage programs, with down regulation of

Please cite this article in press as: Povinelli, B.J., et al., Single cell analysis of normal and leukemic hematopoiesis, Molecular Aspects of Medicine (2017), http://dx.doi.org/10.1016/j.mam.2017.08.006