LETTER

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Clonal analysis of lineage fate in native haematopoiesis

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Haematopoiesis, the process of mature blood and immune cell production, is functionally organized as a hierarchy, with self-renewing haematopoietic stem cells and multipotent progenitor cells sitting at the very top[1,2]. Multiple models have been proposed as to what the earliest lineage choices are in these primitive haematopoietic compartments, the cellular intermediates, and the resulting lineage trees that emerge from them[3–10]. Given that the bulk of studies addressing lineage outcomes have been performed in the context of haematopoietic transplantation, current models of lineage branching are more likely to represent roadmaps of lineage potential than native fate. Here we use transposon tagging to clonally trace the fates of progenitors and stem cells in unperturbed haematopoiesis. Our results describe a distinct clonal roadmap in which the megakaryocyte lineage arises largely independently of other haematopoietic fates. Our data, combined with single-cell RNA sequencing, identify a functional hierarchy of unilineage- and oligolineage-producing clones within the multipotent progenitor population. Finally, our results demonstrate that traditionally defined long-term haematopoietic stem cells are a significant source of megakaryocyte-restricted progenitors, suggesting that the megakaryocyte lineage is the predominant native fate of long-term haematopoietic stem cells. Our study provides evidence for a substantially revised roadmap for unperturbed haematopoiesis, and highlights unique properties of multipotent progenitors and haematopoietic stem cells *in situ*.

To probe native lineage relationships in the fully unperturbed bone marrow, we used the Sleeping Beauty lineage-tracing model and TARIS (T7-amplification mediated recovery of integration sites), an improved transposon integration sequencing technique (Fig. 1a and Extended Data Figs 1 and 2)[11]. Our analysis relies on comparing tags across multiple differentiated populations at different time points to understand the dynamics of lineage coupling, without the need to isolate and transplant prospective progenitor subsets (Fig. 1b). We pulsed adult Sleeping Beauty mice with doxycycline (Dox) for two days and, at one, two, four, and eight weeks after induction, sorted transposon-labelled ($DsRed^+$) nucleated erythroblasts, megakaryocyte progenitors (MkP), granulocytes, monocytes, and B-cell progenitors (Fig. 1c). Notably, control experiments demonstrated that negligible amounts of transposition occur one day after removal of Dox (Extended Data Fig. 3).

We observed that blood lineages were mostly segregated for the first two weeks, suggesting their replacement by unilineage progenitors during this period (Fig. 1d). Later, we began to detect a significant number of shared tags across lineages, revealing the activity of oligolineage progenitors (Fig. 1d and Extended Data Fig. 4). At four weeks, 40.5% (±8.4%) of all monocyte-detected tags (approximately 289 ± 89 clones) were also found in the granulocyte compartment, confirming their well-established common origin (Fig. 1e)[4]. Unexpectedly, a similar proportion of erythroblast clones were also found shared with granulocyte/monocyte (myeloid) tags (Fig. 1d, e), revealing a common progenitor for erythrocytes, granulocytes, and monocytes at this stage. Remarkably, we detected virtually no MkP clones that were shared exclusively with erythroblasts during the whole period of observation, which would have been predicted had a megakaryocyte–erythroid progenitor (MEP)-like cell existed (Fig. 1d, e and Extended Data Fig. 4b)[12,13]. At eight weeks, our analysis revealed the activity of a set of multilineage clones (239 ± 58), with lymphoid (B-cell progenitor), granulocyte/monocyte, and erythroid contribution, but still with no presence in MkP, indicating the existence of megakaryocyte-deficient lympho-erythromyeloid progenitors (Fig. 1d, e). We did observe a very small (9.7 ± 2.8), yet increasing, number of MkP tags shared with multiple lineages after eight weeks (Fig. 1e and Extended Data Fig. 4a, b), suggesting that clonal megakaryocyte-lineage production can also be associated with multilineage outcomes, although at lower frequencies. Spearman's rank correlation analyses of tag-read distribution between lineage pairs showed a progressive association of granulocyte/monocyte, erythro-myeloid, and lympho-myeloid progenitors, segregated from MkP (Fig. 1f, g). To address potential sampling and sensitivity limitations, we performed independent TARIS amplifications (Extended Data Fig. 5) and clone-specific PCRs (not-shown). Taken together, our results provide evidence for novel lineage couplings during unperturbed haematopoiesis, in which the megakaryocyte lineage is produced largely independently from the other haematopoietic lineages, and argue for the robust activity of erythromyeloid and lympho-erythromyeloid progenitor clones.

We next aimed to identify ancestral relationships by comparing the clonal repertoires of differentiated cells and previously defined progenitor populations. Classically, oligopotent progenitors reside in the common myeloid progenitor (CMP), granulocyte–monocyte progenitor (GMP), and MEP phenotypic gates (referred together as myeloid progenitors, or MyPs)[4]. Our data revealed largely unilineage outcomes for detected MyPs ($89.0 \pm 0.8\%$), suggesting that these populations represent a collection of lineage-restricted progenitors, functionally validating predictions from single-cell expression profiling (Extended Data Fig. 6)[14–16]. We next focused on the multipotent progenitors (MPPs), the cellular subset proposed to be upstream of MyPs. At 1 and 2 weeks, we observed a small number of 'active' MPP tags (overlapping with Lin^+ tags), which aligned mostly with single lineages (1 week: $75.8 \pm 5.0\%$; 2 weeks: $66.3 \pm 6.1\%$), suggesting the existence of a small population of lineage-committed MPPs that rapidly produce differentiated progeny (Fig. 2a, b and Extended Data Fig. 7a). MPP output significantly increased at 4–8 weeks for all lineages ($9.35 \pm 0.6\%$ of all MPP tags at 8 weeks), consisting mostly of oligolineage erythromyeloid clones ($79.2 \pm 5.3\%$ of active MPP clones). A robust number of lympho-erythromyeloid MPP clones (12 ± 2) were detected beginning at eight weeks (Fig. 2a), consonant with our analysis of Lin^+ fractions (Fig. 1f). Although we also observed oligolineage

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