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Bone marrow transplantation experiments

While we confirmed that CD45+ bone marrow derived cells contributed to the dermis during normal homeostasis and wound healing (Fig. E9d, f, h), in contrast to some previous reports, we did not observe any bone marrow derived PDGFRa+ fibroblasts[31-35], even following wounding[36] (Fig. E9e, g, i). We analyzed the entire wound of at least n=5 mice by flow cytometry and never detected GFP expression from the skin of mice that had received PDGFRaH2BeGFP bone marrow transfers (Fig. E9j-k). Our data are in agreement with recent studies showing that the lineage restrictions of the embryonic germ layers are maintained during regeneration of mouse digit tips[35]. Our studies are also consistent with recent studies by Barisic-Dujmovic et al. 2009[37] and Higashiyama et al. 2011[38] that utilized the Col1a1-GFP and the Col1a2-GFP mice. We conclude that resident fibroblast and lymphocytes are separate lineages in the skin that perform different functions.

Clonal growth of fibroblast subpopulations in hydrogels

To examine when lineage commitment occurs we used an in vitro model that supports clonal growth of dermal cells in hydrogels[11]. CD26+, Dlk+Sca1- and Dlk+Sca1+ subpopulations cultured from E16.5 dermis did not spontaneously differentiate into adipocytes but were competent to do so in the presence of adipogenic medium (Fig. E3d-e). In contrast at P2 CD26+ cells were not competent to differentiate into adipocytes, while Sca1+ cells

spontaneously differentiated (Fig. E3f-g). Only Dlk1+Sca1- cells, which exhibited multilineage differentiation in the skin reconstitution assays (Figure 2a-f), remained responsive to adipogenic medium (Fig. E3f-g). These results support the conclusion that lineage restriction occurs at approximately E16.5 of development.

In vivo clonal analysis with PDGFRaCreER

We determined experimentally that 25μg/g injection of Tamoxifen into pregnant female PDGFRaCreERxCAGCATeGFP mice labelled single dermal cells at E12.5 (Fig. E8a-d). We then measured clonal density (number of GFP+ clones per projected surface area of dermis) at E14.5. Clones were detected in all regions of the dermis, although we only present data on papillary clones (Fig. E8e-j).

For the same dose of tamoxifen, the data showed a degree of mouse-to-mouse variation. From a surface area of 1.55x1.55 mm^2, measurements from three mice revealed, respectively, 5, 3 and 3 clones in dermal papillae and 22, 33 and 50 clones in the rest of the dermis. This equates to an average clone density of approximately 15 ± 6/mm^2 of dermis. Projected onto the surface, this translates to a typical clone separation of 260 ± 50 mm, a factor of two larger than the thickness of the dermis. (Note that this estimate does not include the degree of clone separation perpendicular to the layer. Two clones lying one above the other in the plane would be considered coincident.) With an average cell diameter of 17 μm, this means that the

average separation of clones at induction is around 15 ± 3 cell diameters in the plane.

In two dimensions, if the chance of a cell being induced to express GFP is uncorrelated with that of neighbouring cells, the probability of finding a labelled cell within a distance l of another at induction is given by $P(l)=1-\exp[-(l/a)^2]$, where a denotes the average clone separation. Therefore, to estimate the chance of merger events, we have to decide upon the effective value of l beyond which clones can be reliably discriminated. Clearly, if two neighbouring cells are labelled at induction, it will be impossible to resolve them as independent clones. Indeed, with the estimate above, this probability can be considered as unfeasibly small. However, in the present case, we have to be more conservative. In the course of development, there is a considerable degree of cellular migration of the progeny of the clone as the tissue matures forcing us to revise upwards the estimate of l. If we assume that cells separated by a distance comparable to the tissue thickness represents a reasonable in-plane separation beyond which clones can be clearly discriminated, we are led to an estimate of around $l/a=1/2$, i.e. in this case, the chance of clone merger is around 0.22. This means that some 20% (i.e. 1 in 5) of recorded clones can be attributed to unresolved merger events.

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