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Current Opinion in Cell Biology

Clonal analysis of stem cells in differentiation and disease

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Tracking the fate of individual cells and their progeny by clonal analysis has redefined the concept of stem cells and their role in health and disease. The maintenance of cell turnover in adult tissues is achieved by the collective action of populations of stem cells with an equal likelihood of self-renewal or differentiation. Following injury stem cells exhibit striking plasticity, switching from homeostatic behavior in order to repair damaged tissues. The effects of disease states on stem cells are also being uncovered, with new insights into how somatic mutations trigger clonal expansion in early neoplasia.

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Current Opinion in Cell Biology 2016, **43**:14–21

This review comes from a themed issue on **Differentiation and disease**

Edited by **Tom Misteli** and **Graham Warren**

http://dx.doi.org/10.1016/j.ceb.2016.07.002

Introduction

Many adult tissues are continually turned over. New cells must be made at a rate that exactly matches cell loss. This balance is critical, as if slightly too few cells are made the tissue will fail while excess cell production is a feature of cancer. New cells of each lineage are produced by stem cells. Clonal analysis to resolve the fate of individual rather than bulk populations of stem cells has revealed the cellular mechanisms by which stem cells sustain a variety of lineages throughout life. The proliferative diversity between tissues and the dynamic and adaptable nature of the cells that sustain them makes defining the term 'stem cell' ever more challenging. Here we will adopt a purely functional definition, stem cells are cell populations that maintain and/or regenerate adult tissues or lineages [1,2]. We discuss the rapidly developments in clonal analysis in three adult stem cell systems, intestine, squamous epithelium and blood, and consider how recent results have revised the stem cell paradigm.

Stem cells in homeostasis

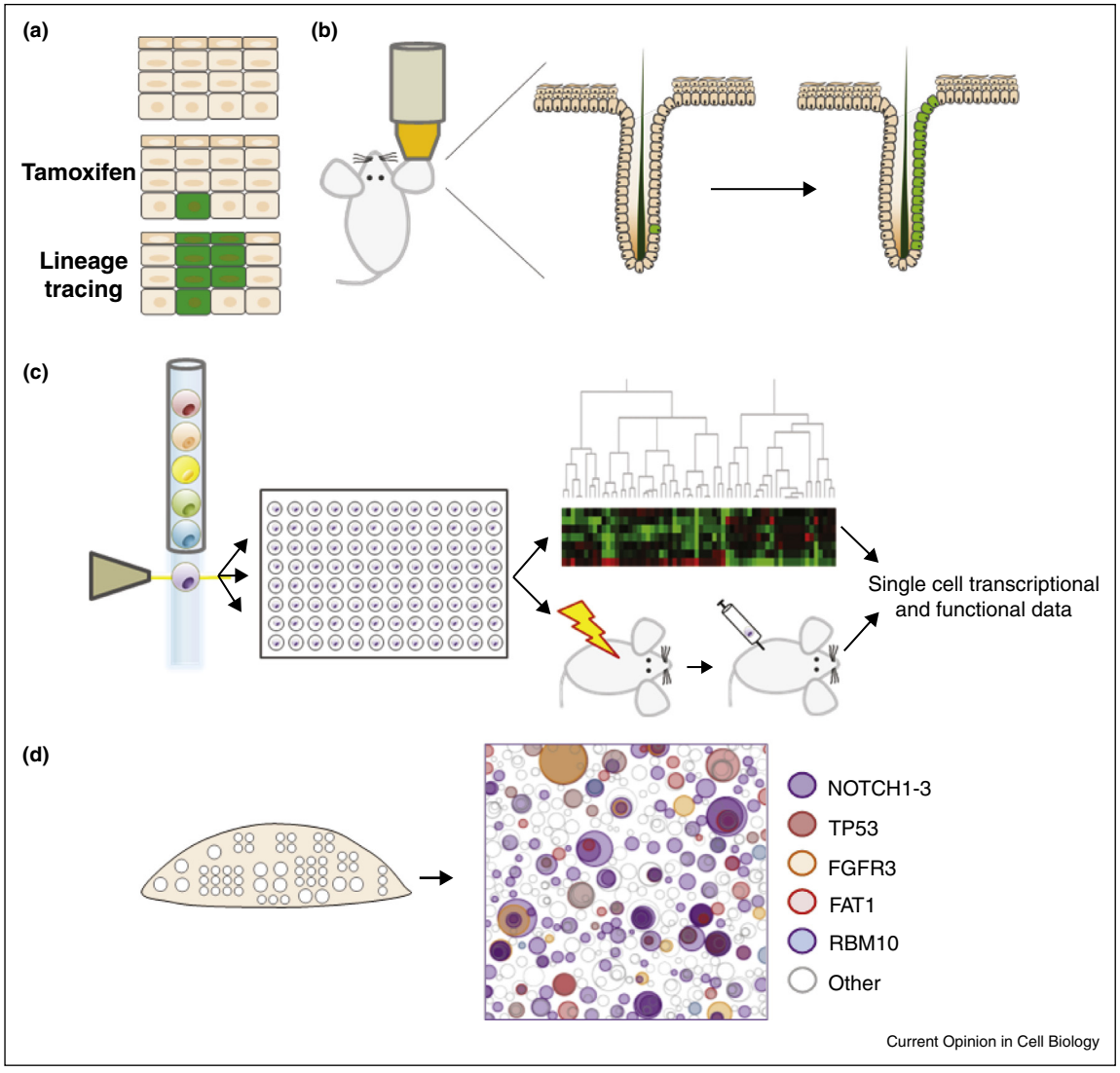
Intestinal epithelium

Our understanding of the stem cells of the epithelium that lines the intestine has been transformed by the application of clonal analysis in transgenic mouse models (Figure 1a, b) [3]. The tissue is rapidly turned over. Differentiated cells on the finger-like villi are continually shed and replaced by proliferating cells located in pits, known as crypts, which lie adjacent to the villi [4]. Inducible genetic lineage tracing of single cells expressing the *Wnt* target gene *Lgr5* reveals clones containing the four differentiated cell lineages of the epithelium, some of which persist long term (Figure 2a) [5]. This indicates that the Lgr5+ population both sustains itself and maintains the epithelium. *In vitro* clonal analysis, in which LGR5+ cells were cultured revealed that the progeny of single cells could self assemble into intestinal like 3 dimensional structures termed organoids that contained the four differentiated cell types and could be serially propagated, as long as the media contained *Wnt* ligands [6]. *In vivo*, the source of WNT is the Paneth cells that lie adjacent to the *Lgr5*+ cells at the crypt base (Figure 2a). [7]. Fluorescent tagging of WNT3 in a transgenic mice reveals that the restricted distribution of WNT signaling at the crypt base is due to the protein remaining bound to cell membranes and being diluted when cells divide [8••]. This mechanism restricts stem cells to the crypt base, as once they leave the niche, stem cells receive less WNT signal and undergo differentiation [9,10]. In each crypt stem cells compete neutrally with their neighbours, with the result that, purely by chance, the crypt will eventually become colonized by the progeny of one stem cell [9,10]. It was thought that all Lgr5+ cells contributed equally to tissue maintenance, but more recent studies have shown that only a third of the cells in the crypt are proliferating at any one time. Combined intravital imaging with genetic lineage tracing has shown that the cells in the uppermost part of the niche are the most likely to differentiate [11].

Squamous epithelia

The outermost layer of the skin, the epidermis, and the lining of the oesophagus consist of layers of keratinocytes (Figure 2b, c) [2,12]. Proliferation is confined to the basal layer of cells. On commitment to terminal differentiation, cells exit the cell cycle and leave the basal layer, migrating to the tissue surface from which they are shed. There are conflicting models of how the epidermis is maintained.

Figure 1



Methods of clonal analysis. **(a)** In this example, genetic lineage tracing in the epidermis is activated by tamoxifen induced cre recombination, leading to the reporter gene (GFP, green) being expressed in scattered cells in the basal cell layer. Expression of the label is inherited by the progeny of the labelled cell (green), revealing the fate of the labelled cell and its daughters over the time since induction. **(b)** Lineage tracing can be combined with intravital imaging to study stem cell biology in live animals, for example in tracking single stem cells within the hair follicle during homeostasis and regeneration. **(c)** Index sorting allows parallel transcriptional and functional analysis of individual cells in a population sharing the same surface markers and may reveal unexpected heterogeneity at the single cell level. **(d)** Deep targeted exome sequencing of small samples of normal sun-exposed human skin has revealed a high burden of clones carrying oncogenic driver mutations (represented by shaded circles). Measured clone areas are projected onto a simulated 1 cm^2 area of skin, open circles indicate neutral mutations.

Statistical analysis of inducible genetic lineage tracing argues that the proliferating cells in the basal layer of the epithelium contribute equally to tissue maintenance [13–17]. The outcome of individual cell divisions is unpredictable, producing two differentiating daughters, two dividing cells or one cell of each type. However, the probabilities of generating dividing or differentiating daughter cells are balanced so homeostasis is achieved across the population of dividing cells. The case for this single progenitor model has been strongly reinforced by a recent study of ear and paw epidermis which combines intravital imaging with transgenic lineage tracing to show that there are no slow cycling stem cells at these sites and that measurement of proliferating cell behavior is entirely consistent with a single cell type [18••]. It appears that not all body sites are the same however. Tail epidermis does contain a slow cycling stem cell population in addition to progenitors that is mobilized following injury [19]. Another report fails to find evidence for slow cycling cells in back skin but argues, in contradiction to earlier work, that there are two populations of rapidly dividing progenitor cells dividing at different rates in different regions of the