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Review

Act your age: Tuning cell behavior to tissue requirements in interfollicular epidermis

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ABSTRACT

In all tissues the balance of cell proliferation and differentiation needs to be tuned to match the varying requirements of embryonic development and adult life. This is well illustrated by the interfollicular epidermis (IFE), which undergoes expansion and remodeling in utero, significant post natal growth and is then maintained in homeostasis. In addition to sustaining a high daily turnover of cells, the epidermis is able to re-populate areas of tissue damage due to common environmental stresses such as wounding. Here recent insights into proliferating cell behavior in IFE and how this changes through development and into adulthood are discussed.

Contents

1. Introduction

The mammalian epidermis is a remarkable tissue, forming a flexible but impermeable surface barrier from embryonic development throughout life. The interfollicular epidermis (IFE) consists of multiple ordered layers of keratinocytes overlying a basement membrane and is punctuated by appendages such as hair follicles and sweat glands. Even in adulthood the epidermis has to keep "running to stand still", being in a constant state of flux as differentiated cells are continually shed from the external surface while new cells are generated in the basal layer. In addition, because the epidermis is continually subject to injury, it must be able to repair itself to restore this protective barrier. It has recently become possible to resolve the behavior of proliferating cells in vivo in the epidermis of transgenic mice. This review considers advances in tracking cell fate through lineage tracing, which are starting to reveal how the proliferating cell behavior is tuned to meet tissue requirements in IFE at different life stages.

2. Development: growing thicker and larger

During development the epidermis develops from a single layered, fragile and permeable surface ectoderm into a multilayered structure that forms an impermeable barrier to the external environment [1,2]. In the mouse embryo, the earliest recognized molecular sign of surface epithelial development is the expression of the simple keratins KRT8 and KRT18, and the induction of the epidermal lineage-determining transcription factor p63 at E8.5 through dermal signaling [3,4]. At approximately E9.5, the

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basal keratinocyte identifiers KRT5 and KRT14 are expressed prior to the first morphological change seen with the formation of an intermediate cell layer between the basal layer and periderm [5–7]. The intermediate keratinocytes express the differentiation associated keratin KRT1 and continue to divide, such suprabasal layer proliferation is a feature that appears to be unique in this short developmental window. Following cell cycle exit, intermediate layer cells withdraw from cell cycle and form post-mitotic suprabasal keratinocytes [3,6]. By birth, the epidermis is an ordered multilayered structure of dividing basal keratinocytes continuing to express KRT5 and KRT14, overlaid by non-dividing suprabasal spinous layer keratinocytes expressing KRT1 and KRT10, which progress through granular layers as terminal differentiation proceeds, eventually being shed from the cornified outer layer.

A keen focus of developmental studies on IFE has been the orientation of mitoses in the basal layer of embryonic epidermis. When basal cells divide they may do so either with the mitotic spindle parallel with the basement membrane, generating two basal cell daughters (planar division, parallel with the basement membrane) or perpendicular to the basement membrane, producing one basal cell and one suprabasal cell (a perpendicular division, Fig. 1). Prior to E12.5 mitoses are predominantly planar [6]. The proportion of perpendicular divisions then increases from <10% at E12.5 to peak at 90% as the intermediate cell layer forms [6,8,9]. The proportion of perpendicular mitoses then falls progressively, falling to 35% at birth, until adulthood when almost all divisions are planar [6,10,11] (Fig. 1). Additionally the frequent suprabasal planar divisions characteristic of early epidermis are absent in later development and adulthood.

The regulation of mitotic orientation in developing epidermis has been the subject intensive research and the focus of several excellent reviews, so we will not comment on it in depth, but it is worth highlighting one key point [9,12–14]. Perpendicular divisions are sometimes referred to as asymmetric, as the two daughters have different locations, while planar divisions are described as symmetric, implying the daughters are functionally equivalent. However, studies on adult epidermis which is maintained almost exclusively by planar divisions, have revealed the majority of divisions result in daughters with asymmetric fate, with one daughter dividing again while the other stratifies out of the basal layer without further division [10,11,15]. Furthermore, both daughter cells from a perpendicular division during development may be proliferative, one dividing in the basal layer, the other in the intermediate layer [6]. Using terminology equating the orientation of mitosis with the fate outcome of daughter cells can thus be confusing. The changes in the proportion of parallel and perpendicular divisions in embryonic IFE cannot be used to infer the proliferative status of the daughter cells and resolve whether there is proliferative heterogeneity in development.

Tracking the outcome of cell divisions in embryonic epidermis is challenging as the tissue is expanding rapidly and changing in structure. However, short term lineage tracing experiments tracking genetically labeled cells from E14.5 to E15.5 demonstrate that the progeny of a single basal cell can undergo planar or perpendicular division [9]. This suggests each proliferative cell in the developing epidermis has the capacity to undertake either type of division leading to symmetric or asymmetric proliferative fate, a feature central to adult epidermal homeostasis (see below) [10]. The development of new methods to genetically label IFE cells using lentiviral vectors introduced in utero offers the prospect of lineage tracing combined with genetic manipulation for multiple genes, allows the pathways that regulate cell behavior to be better resolved [12]. In summary, the nature of the proliferating cells which support the development of IFE have yet to be fully resolved, but their net behavior is the production of an excess of cycling over post mitotic cells to sustain the expansion in the surface area and the increase of cell layers from embryonic days E12.5 to E18.5.

3. Adulthood: the challenge of staying the same

In adulthood, the increase in body surface area is halted and the IFE enters a phase of homeostasis where the loss of cells from the epidermal surface matches the production of new cells by proliferation in the basal layer. Despite the apparent simplicity of adult IFE, the nature and behavior of the proliferating cells which maintain epidermal homeostasis has proved controversial.

An early model was based on studies of rat esophagus, a stratified squamous epithelium similar to IFE, in which proliferation is confined to the basal layer and differentiating keratinocytes stratify out of the basal layer eventually being shed at the tissue surface. Cell behavior was followed by administering a pulse of H^3-thymidine to label S phase cells in the basal layer [16]. These went on to divide producing labeled cell pairs. Initially both cells in each pair were, indicating that basal cell division produces two basal cell daughters. However, as labeled cells began to stratify, three kinds of cell pairs containing two basal cells, one suprabasal and one basal cell or two suprabasal cells were observed. Based on relative proportions of each type of cell pair at 48 h, it was argued that all cycling cells were functionally equivalent and had a 50:50 chance of differentiating or going on to divide [16]. Unfortunately the labor intensive nature of reconstructing cell pairs from autoradiogaphy of tissue sections prevented the tracking of sufficient numbers of pairs over a long enough period to confirm this hypothesis.

Later, an alternative model was proposed that has proved highly influential over many years. The "stem/transit amplifying (TA)" cell hypothesis proposed that adult IFE is maintained by long lived, slow cycling, self renewing stem cells, which divide asymmetrically to self renew and generate TA cells [17]. After a limited number of cell divisions, all the progeny of a TA cell undergo terminal differentiation. In the epidermis, it was argued stem and TA cells were arranged into clonal epidermal proliferative units (EPU). Each EPU consisted of a central stem cell, with surrounding TA cells, that maintains the overlying differentiated cell layers. Although the EPU paradigm was widely assumed, it was inconsistent with the findings of a series of studies [11,18,19]. More recently, the advent of *cre* recombinase based inducible genetic labeling in transgenic mice has provided a direct way to track the behavior of proliferating cells in vivo [20,21]. Doubly transgenic mice are engineered to express a drug regulated form of cre and a reporter gene that is only expressed following cre mediated excision of a "STOP" cassette which blocks reporter expression. By using low doses of inducing drugs it is possible to induce reporter expression to label scattered single cells, which subsequently expand into clones with proliferation [10]. Lineage tracing can be combined with wholemount techniques in which pieces of IFE are removed and stained intact [22]. The three dimensional reconstruction of confocal image stacks of wholemount IFE allows entire clones to be visualized at single cell resolution [10,15]. In a tissue such as normal IFE, where there is no detectable apoptosis, the composition of the clone (the number of basal and suprabasal cells) can reveal what happened to the founder cell and its progeny over the time between labeling and analysis. If sufficient clones are analyzed over a prolonged time course, the data can be used to uncover the behavior of proliferating cells.

In a large scale experiment, lineage tracing was applied to adult IFE, first in the specialized epidermis of the tail and subsequently in the more typical epidermis of the ear (Fig. 2a and b). Basal cells were genetically labeled at low frequency with a fluorescent protein reporter, in a cohort of transgenic mice [10,15]. Mice were culled at time points out to a year and hundreds of clones per time point were analyzed by imaging epidermal wholemounts. Clone size increased