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25 YEARS OF EPIDERMAL STEM CELLS

Ruby Ghadially [Professor]

Dept. of Dermatology and Co-Director of the Epithelial Pipeline of the Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, and Veterans Affairs Medical Center, San Francisco

Abstract

This is a chronicle of concepts in the field of epidermal stem cell biology and a historic look at their development over time. The last 25 years have seen the evolution of epidermal stem cell science, from first fundamental studies to a sophisticated science. The study of epithelial stem cell biology was aided by the ability to visualize the distribution of stem cells and their progeny through lineage analysis studies. The excellent progress we have made in understanding epidermal stem cell biology is discussed in this article. The challenges we still face in understanding epidermal stem cell include defining molecular markers for stem and progenitor subpopulations, determining the locations and contributions of the different stem cell niches, and mapping regulatory pathways of epidermal stem cell proliferation and differentiation. However, our rapidly evolving understanding of epidermal stem cells has many potential uses that promise to translate into improved patient therapy.

INTRODUCTION

The last 25 years (1985 – present) have been the time during which epidermal stem cell biology evolved from first fundamental studies to a sophisticated science. The last 25 years have seen an exponential growth in the field of epidermal stem cells. A literature search of “epidermis” and “stem cell” revealed 0 to 5 articles per year in the years from 1975 to 1985, followed by a rapid increase to over 150 articles per year for the last 4 years (Figure 1). In the 60-70’s careful study of epidermal morphology and of cell kinetics gave insight into epidermal proliferation units and of epidermal cell kinetics. This laid a groundwork for our understanding of epidermal stem cells. From the 1980’s to the present our understanding of cutaneous stem cell biology has undergone tremendous progress due to the large body of work that has been conducted, enhanced by knowledge gained from other tissues. This timeline makes the last 25 years a perfect interval in which to journey through and reflect on how our concepts of epidermal stem cells have evolved over time. In Figure 2

Corresponding Author: Prof. Ruby Ghadially, M.B. Ch.B., FRCP(C)Derm Professor, Dept. of Dermatology UCSF, Co-Director Epithelial Section of the UCSF Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research Mailing Address: 4150 Clement St., San Francisco 94121 MC (190) Ph: 415 221-4810 ext 3342 Fax: (415) 750-6959.

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approximations of the occurrence of evolving concepts and scientific evidence for these concepts are illustrated on a timeline.

While the exponential growth is impressive, it can be seen in Figure 3 how growth in the science of epidermal stem cells began approximately 20 years after that in hematopoiesis. It can also be seen that, due to the size of our specialty, the numbers of papers and presumably the volume of work/experiments conducted is of an order of magnitude less than hematopoietic stem cells. However, the bright side is that, following in these steps, we have learned from concepts and knowledge already gained and progressed at an accelerated pace toward a more thorough understanding of epidermal stem cell biology and the ability to use epidermal stem cells for clinical advantage. Furthermore, other fields can learn from the epidermal stem cell field, because skin stem cell work has focused on lineage analysis in tissue sections, allowing visualization of stem cells and their immediate progeny, something bone marrow and blood do not lend themselves to easily.

For this article I have examined the progress of stem cell research from a historical perspective, looking at the evolution of concepts in epidermal stem cell biology over time. In this quest, given the size of the literature and the large amount of progress, I have surely omitted excellent and concept-changing work by many of my epidermal stem cell biologist colleagues, and for this I apologize before I begin.

Till and McCulloch: Hematopoiesis leads the way (1961-)

In 1961 Till and McCulloch published a seminal paper, that was published in its original form again this year, providing a quantitative method for analyzing hematopoietic cells capable of continued proliferation *in vivo* and providing a singularly important observation; that single cells could give rise to all hematopoietic lineages *in vivo* (Till and McCulloch, 1961; Till and McCulloch, 2011; Weissman, 2011). Supralethally irradiated mice were injected with nucleated bone marrow cells and the spleen colony forming units (CFU-S) quantified. The number of macroscopic spleen colonies was directly proportional to the number of cells injected and the colonies were noted to be heterogeneous in size. Further conceptually important experiments studies showed that the clones were heterogeneous in their self-renewal ability (Siminovitch et al, 1963). This was the beginning of quantitative assessment of stem cell proliferation *in vivo* and the quest for methods to study defining characteristics of stem cells – believed to be long term proliferation *in vivo* and self-renewal. Thus, active work in the field of hematopoietic stem cells began almost 20 years ahead of active epidermal stem cell research as can be seen in Figure 3.

Colony formation *in vivo* following skin irradiation (1967-)

The hematopoietic colony forming unit assay from Till and McCulloch determined a dose-survival relationship for injected hematopoietic cells. This was followed by the Withers colony forming assay after skin irradiation, in 1967 (Withers, 1967a; Withers, 1967b). This assay used Poisson limiting dilution statistics to determine the frequency of cells capable of initiating colonies following skin irradiation. Following irradiation small nodules of keratinocytes could be observed by 10-20 days and the colony forming assay allowed determination of the dose-survival relationship (surviving cells per unit area at different

doses of X-rays). This demonstrated the survival of a subset of cells with clonogenic ability post-radiation.

The epidermal proliferation unit – Epidermis is arranged in columns with a precise stacking arrangement and a clonogenic central basal cell (1969-)

The superficial corneocytes, suprabasal cells and basal cells together can be regarded as one epidermal proliferation unit and cells at the base of a cell column produce the more superficial cells. Each epidermal proliferation unit had, on average, 10.6 basal nuclei beneath a column of cells. There are usually 10 - 11 basal cells, 3 flattened nucleated differentiating suprabasal cells, and 5 - 7 cornified cells making a total of 18 - 21 cells spanning on the order of 0.05 millimeter (Mackenzie, 1969)(Mackenzie, 1970). Thus, in the epidermis of many body regions of several species there is a basic structural unit composed of approximately 20 cells.

Potten and Hendry reviewed data from three studies of keratinocyte survival curves post radiation (Potten and Hendry, 1973). The data showed that the average number of clonogenic cells in murine epidermis ranged from 309 to 1107 clonogenic cells per mm^2. Meanwhile, the number of basal cells in a given area has been reported as 1.4 to 2×10^4 basal cells/mm^2 (Potten and Hendry, 1973). From these data it can be calculated that 1.5% to 14% of basal cells are clonogenic post-radiation. Later, using [3H]thymidine labeling and a one month chase period it was shown that cell division is restricted to the basal cells and the central one may cycle at a slightly slower rate (Morris et al, 1985). In subsequent years, with the success of keratinocyte culture methods, studies of colony-forming efficiency *in vitro* confirmed that the frequency of clonogenic keratinocytes in culture also ranges from 2 – 8% in mouse (Morris et al, 1988; Bickenbach and Chism, 1998; Popova and Morris, 2004). Thus, there is good evidence that epidermal proliferation units have a clonogenic cell at their origin.

Morphologic definition of these proliferative units in epidermis greatly aided our understanding of cell replacement in interfollicular epidermis, while the issue of the contributions of the hair follicle stem cells to interfollicular epidermis was only to be addressed at a later date.

Lajtha: A hierarchy of proliferative epithelial cells (1979)

A thoughtful outline of concepts of stem cell proliferation and differentiation succinctly summarized, including the concept of a hierarchy of proliferative epithelial cells, with only a small fraction of them acting as stem cells. This work remains much read and regularly cited today (Lajtha, 1979).

Label retaining cells as stem cells in murine epidermis (1981-)

While human studies of epidermal stem cells have been largely restricted to *in vitro* colony studies, studies in mice have assayed label-retaining cells as stem cells. Mice were continuously or pulse labeled, with either tritiated thymidine or 5-bromo-2’-deoxyuridine (BrdU), to generate labeled cells and then the kinetics of accumulation/loss of label examined (Morris et al, 1985; Potten, 1974; Bickenbach, 1981; Bickenbach et al, 1986;

Potten and Morris, 1988). These studies relied on the concept that an epidermal stem cell might be identified by a slow cell-cycle duration. Injections of nucleotide analogues were given repeatedly at a time when the tissue is hyperproliferative, so that all dividing cells were labeled. Weeks to months later, cells that rarely divide and therefore retain their label were considered stem cells (Morris et al, 1985; Bickenbach, 1981; Bickenbach et al, 1986). Slower cycling cells, in the center of epidermal proliferation units, could be identified using label retaining ability, in contrast to the more rapidly proliferating cells located in the peripheral region of the epidermal proliferation unit that do not retain DNA label (Bickenbach, 1981). The number of labeled mitoses in the epidermis examined over time indicated the existence of at least two distinct populations of cells with different cell cycle durations, as well as a post-mitotic population (Potten and Morris, 1988). Later (1990), it was shown that in murine hair follicle, label retaining cells were confined to the bulge region (Cotsarelis et al, 1990).

The issues with this method include the concern that since very few stem cells are dividing at any time only a small subset of stem cells would be labeled and the most primitive stem cells may remain unlabeled. Another issue was that after damage caused by incorporation of a nucleotide analogue the cells may not be capable of dividing. This however, does not appear to be the case, as label-retaining cells have been stimulated to divide (Bickenbach, 1981)(Braun et al, 2003). Also in more recent studies fluorescently labeled slow-cycling cells were produced in a keratinocytes. This method was used to purify the label-retaining cells that mark the skin stem cell niche and to follow the progeny of label retaining cells in the murine bulge (Tumbar, 2004). For excellent reviews of label retaining epidermal cells see Braun and Watt (Braun and Watt, 2004) and Fuchs (Fuchs, 2009).

Clonal analysis of human epidermal stem cells establishes a hierarchy of proliferative keratinocytes (1987-)

Study of human epidermal stem cells has been largely based on studies in short-term *in vitro* culture. In an effort to identify cells with a high proliferative capacity in human epidermis Barrandon and Green studied the self-renewal ability of human epidermal cells in replating assays (Barrandon and Green, 1987). For clonal analysis of a population of keratinocytes, 50-100 cells were individually plated. More than 50% of cultured human keratinocytes formed colonies. Six days later colonies were resuspended and each plated into a 100 mm Petri dish. Different cell types founded different types of colonies in secondary culture (Barrandon and Green, 1987). Cells that form mostly large smooth colonies with less than 5% of small abortive colonies are termed holoclones. Paraclones are terminally differentiating cells that form small and abortive colonies. Cells intermediate between stem and differentiated cells form meroclones, intermediate in appearance and reproductive capacity. In the studies of Barrandon and Green (Barrandon and Green, 1987) cultured keratinocytes produced 28% holoclones, 49% meroclones, and 23% paraclones. It is believed that the holoclones are stem cells and that the paraclones are transit amplifying cells (Barrandon and Green, 1987), thereby suggesting a hierarchy of epidermal stem cells beginning with an epidermal stem cell which gives rise to a continuum of cell populations with progressively diminishing capacity to proliferate and self-renew. These studies provided a new way to examine for keratinocytes capable of self-renewal *in vitro.*

Isolation of a nearly pure population of murine hematopoietic stem cells (1988-)

Mouse bone marrow hematopoietic stem cells were isolated with the use of a variety of phenotypic markers. Thirty of these cells were sufficient to save 50 percent of lethally irradiated mice, and to reconstitute all blood cell types in the survivors (Spangrude et al, 1988; Weissman, 2002). Being able to obtain a nearly pure population of stem cells was key to more rapid progress in the understanding of hematopoietic stem cell self-renewal and differentiation.

The bulge, not the bulb, is the site of hair follicle stem cells in the mouse (1990-)

It was previously believed that hair follicle stem cells resided in the bulbar area of hair follicles. However, studies in the 60's had shown that after surgical removal of the matrix/ hair bulb an entire hair follicle could still be regenerated (Oliver, 1966a; Oliver, 1966b). While the bulbar region of the hair follicle was known to contain a pool of relatively undifferentiated epithelial cells, termed matrix cells (that give rise to the hair and the inner root sheath), studies of label retaining cells in mice indicated that cells responsible for the cyclical regeneration of the lower follicle are located in the hair follicle bulge (Cotsarelis et al, 1990). Furthermore, when rat follicles were microdissected that cells from the bulge had the highest colony forming efficiency (Oshima et al, 2001). These studies established that the bulge is a site of murine hair follicle cells capable of cyclic regeneration of the hair follicle.

Colony forming efficiency as a surrogate test for stem cell behavior (1993-)

In vitro studies showed that the population of cultured keratinocytes that adheres most rapidly to collagen IV is enriched in colony forming cells (Jones and Watt, 1993). Whether this population of rapidly adherent cells includes a greater proportion of holoclones was not studied. In rats, 95% of colonies formed in culture were from cells of the vibrissa bulge region (Kobayashi et al, 1993). In human hair follicles, the greatest colony forming efficiency ability was in the lower outer root sheath (Rochat et al, 1994), while label retaining cells were localized in the human bulge area (Lyle et al, 1998). The stage of the human follicle at the time of isolation may influence colony forming efficiency in humans, given that hair follicles are not synchronized in their growth (Rochat et al, 1994) (Oshima et al, 2001). Proliferation *in vitro*/ colony forming ability became a popular surrogate assay by which to analyze for the stem cell like nature of a population of cells, [for example (Braun and Watt, 2004; Tani et al, 2000; Inoue et al, 2009; Nijhof et al, 2006; Ohyama et al, 2006)], while the more arduous method of clonal analysis for self-renewal ability was less utilized.

The search for a molecular signature for epidermal stem cells begins (1993-)

Jones and Watt showed that high levels of β1 integrins on the surface of human epidermal keratinocytes correlated with high proliferative potential *in vitro* (Jones and Watt, 1993). This work was followed by an extensive amount of work by many groups with the goal of isolating a keratinocyte population enriched for epidermal stem cells. Since functional assays to identify stem cells were lacking for epidermal stem cells, surrogate assays including colony formation, cell cycle status, cell size, etc. were used. As a result an extensive number of strategies have been proposed to enrich for epidermal stem cells, but

controversy exists regarding the best epidermal stem cell marker and the degree of the enrichment provided by each method is unknown.

A summary of many of the putative epidermal stem cell markers is included in Table 1. The results from these various studies are difficult to compare as different populations of cells (human, murine, follicular, interfollicular, non-follicular/glaborous) were studied using different methods. Only quantifying the degree to which functional stem cells are isolated by these different methods, through the use of a standardized approach, will allow the molecular signature of the epidermal stem cell to be determined, and thus provide us with the best approach(es) for enriching populations of epidermal stem cells. Based on findings in the hematopoetic system we can anticipate that a combination of mutiple positive and negative surface markers will be required to identify the epidermal stem cell.

The hair follicle bulge as a site of hair follicle stem cells in humans (1996-)

The bulge is a less morphologically distinct structure in the adult human compared to the adult mouse. However, studies of label-retaining cells indicated that human hair follicle stem cells were located in the bulge of the human hair follicle (Lyle et al, 1998; Ohyama et al, 2006). Human bulge cells were found to express Keratin 15 and Keratin 15 expressing human keratinocytes were slow cycling, proliferated at the onset of follicle growth, expressed a high level of B1 integrin (Lyle et al, 1998; Lyle et al, 1999). Moreover, bulge cells isolated by laser capture microdissection were highly clonogenic *in vitro* (Ohyama et al, 2006). Recently, a cell surface marker, CD34, was reported as a specific marker of murine bulge keratinocytes (Trempus et al, 2003). CD34 positive cells were predominantly in G0/G1, and had higher α6 integrin expression than CD34 negative cells (Trempus et al, 2003). However, CD34 was not expressed in human bulge cells (Ohyama et al, 2006; Cotsarelis, 2006a). Having confirmed the bulge as a site of hair follicle stem cells in humans, in a recent study, human bulge cell markers were used to determine that human hair follicle stem cells were maintained in men with androgenetic alopecia, but that CD200-rich and CD34 positive progenitors were decreased (Garza et al, 2011).

Not all colony forming cells are stem cells (1998-)

Phenotypic analysis of hematopoietic stem cells provided the ability to separate long-term proliferating cell from cells detected in colony forming assays (Hodgson and Bradley, 1979; Van Zant, 1984; Lerner and Harrison, 1990; Spangrude and Johnson, 1990; Morrison and Weissman, 1994; Weissman, 2002; Trevisan and Iscove, 1995; Randall et al, 1996). Hematopoietic stem cells have also been shown to have minimal to no clonal capacity (Sutherland et al, 1990; Haylock et al, 1992). While tissues may differ in their biology, it behooves us not to ignore the knowledge obtained through extensive and thorough research in hematopoiesis.

Concerns with the use of colony forming efficiency to assess epidermal stem cells include that 1) culture conditions commonly used have been designed to produce cells as fast as possible in the short-term and therefore may favor proliferation of committed progenitors; 2) cell culture may affect cell division creating an alteration in the number of stem cells (for review see (Cotsarelis, 2006b) 3) removing cells from tissue for *in vitro* analysis requires

perturbing the system so that some events may be the result of a response to wounding and not homeostasis (Kaur, 2006); 4) the population of keratinocytes enriched in short-term colony forming cells (rapidly adherent to collagen IV) *in vitro* contains significantly fewer long-term repopulating cells *in vivo* compared to the population of keratinocytes not enriched in colony forming cells (not rapidly adherent to collagen IV) (Strachan et al, 2008) and; 5) there is prior evidence that stem cell-rich fractions exhibit substantially lower CFE than the source total population in adult rat epidermis (Pavlovitch et al, 1991), neonatal human foreskin (Li et al, 1998) and limbal epithelium (Budak et al, 2005; Selver et al, 2011).

Whether colony forming units reflect true long-term functional capabilities in the epidermis is not known, but it seems likely that long-term repopulating stem cells are either a subset of, or a different set of cells from, the colony-forming cell. Thus the majority of colony-forming cells in short-term cultures may represent intermediate but committed progenitors rather than true epidermal stem cells (given their relatively high frequency and high level of cell cycling). All together these observations indicate that *in vitro* colony forming assays are not an adequate surrogate for *in vivo* assays and much work is still needed to understand how *in vitro* growth relates to epidermal progenitor type and stem cell behavior *in vivo.*

Notch signaling is important, both for cell-fate decisions and in lineage commitment (2000-)

Notch signaling is important in determination of stem cell self-renewal versus differentiation. Signaling via the Notch receptor, delivered by ligands Delta and Serrate, plays a key role in cell fate decisions in both Drosophila and vertebrate development (Artavanis-Tsakonas et al, 1995). In human epidermis expression of Delta (Notch ligand) in stem cells has been proposed to induce differentiation of the neighboring (Notch1 expressing) cells (Lowell et al, 2000). Expression of the Jagged 1 and 2 ligands and Notch 1 and 2 receptors increases in differentiating keratinocytes of the suprabasal layers and is thought to be important for synchronization of differentiation and epidermal border formation (Luo et al, 1997)(Rangarajan et al, 2001). The deletion of the Notch 1 and 2 and/or RBP-Jκ genes indicated a role for Notch signaling in lineage commitment along the hair follicle lineages versus interfollicular lineage (Yamamoto et al, 2003; Pan et al, 2004; Blanpain et al, 2006). For two comprehensive reviews see (Dotto, 2008; Watt et al, 2008).

Evidence for an interfollicular stem cell: Lineage tracing *in vivo* - Seeing is believing (Table 2) (2001-)

While the ability to illuminate epidermal clones with retroviral transduction of keratinocytes was elegantly demonstrated in 1997 (Mackenzie, 1997), it was a few years before evidence for a long-term repopulating cell in the interfollicular epidermis was provided by *in vivo* lineage tracing (Ghazizadeh and Taichman, 2001; Niemann and Watt, 2002; Schneider et al, 2003; Ghazizadeh and Taichman, 2005; Ito et al, 2005; Langton et al, 2008). In these types of studies a label is integrated into the cell's genome and this label is stably inherited by all progeny. Such cell fate mapping techniques allow investigation of the distribution of progeny arising from a single precursor cell. Prior to this time there was uncertainty whether the interfollicular stem cell represented a true stem cell or an early progenitor derived from

follicular stem cells. However, with the ability to perform genetic label lineage tracing studies strong evidence was provided that under homeostatic conditions an independent pool of stem cells maintains the interfollicular epidermis. Previous studies of label retaining cells had also indicated that interfollicular epidermis is maintained by a stem cell compartment distinct from the follicle stem cell compartment (Braun et al, 2003). Cleverly designed studies to ablate hair follicles in hairy skin provided further evidence for an independent stem cell population (Ito et al, 2005). Finally, although many studies show that hair follicle-derived stem cells participate in acute wound closure, analysis of wound healing in the Edaradd mutant mouse (with developmental defects in hair follicle formation) showed that hair follicle stem cells are important but not necessary for normal wound healing or interfollicular epidermis homeostasis (Langton et al, 2008).

There is also evidence for multipotency of ‘interfollicular’ stem cells. There is evidence that hairless skin possesses pluripotent stem cells that, under the appropriate conditions, can produce hair follicles. Even though there are no hair follicles in the foreskin (the source of human keratinocytes for most *in vitro* studies), implantation of dermal papillae results in hair development from keratinocytes of these non-hairy sites (Ehama et al, 2007). It should be noted however, that these studies investigate whether non-hairy skin, not interfollicular skin, is multipotent. The question of whether interfollicular cells are multipotent is somewhat different, since presumably the non-hairy/glaborous skin contains a primitive stem cell, while perhaps (if the pluripotent epidermal stem cell in hairy epidermis is restricted to the hair follicle) the interfollicular epidermis may not. Thus, while non-hairy skin is multipotent, interfollicular epidermis may or may not be. This issue is germane for stem cell isolation and gene therapy. However, together, the above data provide strong evidence for the presence of an distinct stem cell in the interfollicular epidermis.

Long term repopulation *in vivo* as a functional assay of epidermal stem cells (2003-)

It is evident that methods to evaluate epidermal stem cells are inadequate and long-term repopulation assays that measure sustained epithelial tissue regeneration, analogous to “gold standard” assays in hematopoiesis, are essential for progress in epidermal stem cell biology.

Dr. Kaur’s group designed a transplantation assay based on repopulation of epidermis inside a rat trachea implanted subcutaneously in a SCID mouse (Li et al, 2004; Pouliot et al, 2005). This assay allows long-term maintenance of transplanted epidermal cells *in vivo*.

Similarly, our group developed a *in vivo* transplantation assay for epidermal stem cells (Schneider et al, 2003) based on well-established functional assays for hematopoietic stem cells (Chang et al, 2000; Harrison, 1980; Szilvassy et al, 1990; Taswell, 1981). This assay was combined with limiting dilution analysis to enable the quantization of the frequency of long term repopulating cells in a population of keratinocytes.

Using long-term repopulation combined with a limiting dilution design, stem cell frequency in the bone marrow was determined to be 1 in 10,000 nucleated cells (Szilvassy et al, 1990; Taswell, 1981). This type of assay is now a standard in hematopoiesis, for the evaluation of stem cell markers (Weissman, 2002; Spangrude et al, 1988) and for evaluation of stem cell differentiation and regulation (Mikkola et al, 2003; Park et al, 2003; Smith et al, 1991) and

has been key in studying other populations of hematopoietic stem cells quantitatively (e.g. aging/ disease/ cancer) (Chen et al, 1999; Dick, 2003; Lapidot et al, 1994; Sudo et al, 2000). Similar *in vivo* transplantation assays showed that the frequency of long term repopulating mammary gland stem cells was 1 in 4,900 lin^- cells (Shackleton et al, 2006).

The *in vivo* transplantation assay for epidermal stem cells allows similar - quantization of epidermal stem cells. This assay has been used to demonstrate that the frequency of murine epidermal stem cell in this model is less *in vivo* than previously predicted and more in line with hematopoietic stem cell frequency, on the order of 1 in 10,000 (Schneider et al, 2003), that rapidly adherent colony forming cells are not enriched in long-term repopulating cells (Strachan et al, 2008) and while no significant difference in aged versus young epidermal stem cell (long-term repopulating cell, 9 weeks of repopulation) frequency could be detected, transient amplifying cell (short-term repopulating cell, 3 weeks of repopulation) frequency was greater in the aged (Charruyer et al, 2009).

Epidermal Immigrants. Bone marrow derived stem cells in the epidermis during epidermal regeneration (2004-)

Studies have shown that with regeneration, bone marrow derived cells not only contributed to dermal but also epidermal cells (Fathke et al, 2004; Deng et al, 2005; Brittan et al, 2005; Badiavas et al, 2003; Borue et al, 2004). Although it was suggested that bone marrow derived cells could form fusion cells with keratinocytes in an *ex vivo* study (Baxter et al, 2004), other studies indicated that fusion does not occur *in vivo* (Borue et al, 2004; Wu et al, 2007; Harris et al, 2004) and indicate that that bone marrow derived stem cells differentiate into epidermal cells. For a detailed review see Wu, Y et al (Wu et al, 2010).

2004-Stem cell differentiation: p63 is responsible for initiation of epithelial stratification

One protein that is critical for transit amplifying cell regulation is p63. It has been suggested that ΔNp63 is involved in maintaining transit amplifying cells and in preventing premature onset of differentiation (Truong et al, 2006; Honeycutt et al, 2004; Koster et al, 2005) and ΔNp63 was found to be important for maintaining the proliferative state of transit amplifying cells (Truong et al, 2006). p63 is strongly expressed in epithelial cells with high clonogenic and proliferative capacity (Senoo et al, 2007) and mice with reduced ΔNp63 expression show excessive keratinocyte proliferation while lacking normal differentiated layers (Koster et al, 2007). The role of p63 in epidermal stratification is discussed in detail in a review by Dr. Koster (Koster, 2009).

2005- Expansion versus maintenance of the epidermis – symmetric versus asymmetric stem cell division

Stem cells in the niche undergo symmetric self-renewal divisions leading to two daughter cells identical to the original cell, symmetric differentiation divisions releasing two committed cells, and asymmetric self-renewal divisions leading to one daughter cell identical to the original stem cell and another non-stem daughter cell that leaves the niche and undergoes differentiation (Xie and Spradling, 2000; Potten and Loeffler, 1990). When stem cells divide asymmetrically, tissues maintain a constant number of stem cells while, depending on the division rate, allowing a progressive increase in the number of transient

amplifying cells. Symmetric self-renewal divisions allow maintenance and/or expansion of the immature precursor pool and symmetric differentiation divisions allow only differentiated progeny to be generated.

In *Drosophila*, during asymmetric division (using an intrinsic mechanism), stem cells set up an axis of polarity during interphase that orients the mitotic spindle and ensures the asymmetric segregation of self-renewal determinants (for review see (Horvitz and Herskowitz, 1992; Knoblich, 2008; Lechler and Fuchs, 2005). A second (extrinsic) mechanism places the axis of polarity perpendicular to the basement membrane so only one daughter cell remains in contact with the stem cell niche and maintains the ability to self renew. In this case the basal cell remains attached by integrins to the basement membrane (Blanpain and Fuchs, 2009). These two types of asymmetric division have been observed both in epidermal stratification during development and homeostasis of adult epidermis (Lechler and Fuchs, 2005). The extrinsic mechanism is observed in adult stem cells mainly, while intrinsic (and parallel to the basement membrane asymmetric division is more common during epidermal development [for review see (Knoblich, 2008; Blanpain and Fuchs, 2009)].

Although involvement of mitotic spindle orientation in asymmetric fate has yet to be determined, the ability of spindle orientation to change during epidermal development has been shown in epidermis (Lechler and Fuchs, 2005). Basal progenitors first divide in a single layer parallel to the basement membrane. After, during epidermal stratification, they divide perpendicular to the basement membrane. The increased proportion of perpendicular cell divisions in the late versus early embryonic stages allows a balancing of the generation of suprabasal layers with lateral skin expansion. In adult skin the proportion of this asymmetric division is thought to decrease (Lechler and Fuchs, 2005). Recently it was shown that compromising asymmetric cell divisions results in profound defects in stratification, differentiation and barrier formation and Notch signaling is implicated as an important effector (Williams et al, 2011).

Understanding the different mechanisms used by stem cells to divide and proliferate will allow the development of strategies by which to correct diseases such as cancer and psoriasis, in which normal stem cell divisions may be altered.

2007- A single epidermal progenitor versus a hierarchy of progenitors

Proliferating cells of the epidermis have been shown to be heterogeneous, by presence of a subset of label retaining cells (Braun and Watt, 2004), the association of actively cycling cells and terminal differentiation (23), and the heterogeneity of clonal growth in culture (Barrandon and Green, 1987). Classically the epidermis has been thought to be sustained by an epidermal stem cell/transient amplifying cell hierarchy of progenitors, in which the epidermal stem cell divides only occasionally (asymmetrically) to give rise to both a daughter stem cell (identical to the mother stem cell) and a daughter cell called a transit amplifying cell, which terminally differentiates after several rounds of cell division. Recently, however, some observations have contradicted the epidermal stem cell/transient amplifying cell model (Clayton et al, 2007; Jones et al, 2007). Using inducible genetic labeling, the authors were able to trace the fate of progenitor cells in murine tail epidermis *in*

vivo at single cell resolution with a time interval of one year. If the epidermal stem cell/transient amplifying cell model is true and individual stem cells retain their self-renewal capacity, this model would predict that the basal-layer clone size distribution would remain time-independent and follow a "epidermal proliferation unit" pattern. However lineage tracing revealed that the average number of basal layer cells per clone increased with time in a linear manner. Clones remained cohesive and expanded in size over time, such that clone-size distributions were consistent with a new model of homeostasis involving only one type of progenitor cell which may undergo an unlimited number of divisions. If this new model proves correct this will be a major paradigm shift in our understanding of epidermal homeostasis.

2009- Toward regenerative medicine for the epidermis

Although we lack a complete understanding of much epidermal stem cell biology, we already graft both autologous and allogenic keratinocytes for healing of burns and ulcers.

Ideally, rather than culturing autologous cells *in vitro* it might be more effective to expand the pool of epidermal stem cells *ex vivo* and return an expanded pool of stem cells to the wound (Charruyer and Ghadially, 2009). This would be the pool of cells with the greatest proliferative potential for the long-term. Progenitor cells were found superior to more differentiated keratinocytes for generation of tissue-engineered skin (Pellegrini et al, 1999; Dunnwald et al, 2001). Stem cell and transit-amplifying cell populations were used to bioengineer an epidermis in combination with a collagen type I gel containing neonatal mouse dermal fibroblasts. Both populations formed an epidermis, but after two months only the stem cell-derived skin maintained a normal epidermis, while the epidermis formed from transit-amplifying cells had completely differentiated (Dunnwald et al, 2001). A Hoechst and propidium iodide cell-sorting strategy with improved sorting conditions was used to isolate an epidermal stem cell population. This population, when used as a source for tissue-engineered skin, was able to maintain an epidermis and expression of an integrated recombinant gene (Dunnwald et al, 2001). These findings suggest that it may be advantageous to use primitive tissue progenitors for tissue engineering. Finally, successful complete epidermal regeneration on both legs of a patient throughout a 1-year follow-up after transducing primary keratinocytes with laminin B3 cDNA was attributed to transduction of stem cells in the regenerated epidermis (Mavilio et al, 2006; Ferrari et al, 2006).

Another issue is the production of a tissue engineered graft with epidermal appendages. Recent advances are bringing science close to the day where we will be able to produce a three dimensional graft bioengineered graft that contains dermis as well as epidermis with hair follicles and other cutaneous appendages (Marazzi et al, 2011; Mahjour et al, 2011).

2011- Rebuttal to the single progenitor theory from multiple stem cell researchers

For an excellent commentary in response to the single progenitor theory see Kaur and Potten (Kaur and Potten, 2011). Evidence for a proliferative hierarchy in epidermis is based on extensive cell kinetic and mathematical modeling data based on the percentage of labeled mitoses, continuous labeling studies, cell cluster analysis and clonogenic studies following

irradiation. The observed spectrum of keratinocyte behavior could result from a continuum of proliferation ability, as seen in hematopoiesis, and problems identifying transient amplifying cells (committed progenitors) may result from a lack of adequate methods, rather than their non-existence. Finally, the studies of Jones et al were performed on tail skin (Clayton et al, 2007; Jones et al, 2007) and these findings may not be representative of skin from other sites.

2011- From skin to iPs cells and back again: Keratinocytes as a source of iPS cells and iPS cells as a source of keratinocytes

Under appropriate conditions induced pluripotent stem (iPS) cells (derived from fibroblasts or keratinocytes) have the potential to differentiate into to a multitude of cell types. In 2008 iPS cells were generated from human keratinocytes (Aasen et al, 2008) (Carey et al, 2009). Keratinocyte-derived human iPS cells could be differentiated into pancreatic endoderm with an efficiency that was comparable to that for human embryonic stem cells (Santamaria et al, 2010). Recent reports demonstrate that iPS cells can differentiate into keratinocytes (Bilousova et al, 2011; Tolar et al, 2011). The advantages of iPS cells over embryonic stem cells for regeneration therapy include the lack of ethical issues and, since iPS cells can be autologous, the elimination of immune rejection concerns. Another possibility is that iPS cells derived from revertant mosaicism (clinically evident as areas of normal skin in patients with heritable diseases such as epidermolysis bullosa) could be utilized to derive normal iPS cells to provide basically unlimited keratinocytes for grafting (Itoh et al, 2011; Uitto, 2011). Thus, iPS cells provide a novel approach to regenerative therapy for heritable skin diseases such as epidermolysis bullosa. A recent detailed review making a case for iPS cells for regenerative medicine is available (Lemaître et al, 2011).

2011- Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment

While the molecular regulation of specific hematopoietic stem cell properties such as long-term self-renewal is starting to be elucidated for murine HSCs (Sauvageau and Sauvageau, 2010), less is known regarding human hematopoietic stem cells. In a recent study, while CD49f identified hematopoietic stem cells with long-term multilineage potential, loss of CD49f identified transiently engrafting multipotent progenitors. In this study 28% of single $Thy1^{+}Rho^{lo}CD49f^{+}$ cells had long term repopulating ability (Notta et al, 2011). The ability to quantitatively study subsets of hematopoietic stem cells has been a challenging task, involving many hematopoietic stem cell research groups. What we have learned from hematopoiesis will hopefully make the path to quantifying subgroups of epidermal stem cells a shorter one.

Prospects for the future

Surprisingly, the only tissue in which stem cells have been isolated to near purity and *with in vivo* proof of their long-term repopulating ability, is in the hematopoietic system. At present, the isolation of a pure population of epidermal stem cells has not been achieved. Populations of murine bone marrow cells have been enriched so that a purity of 1 in 3 to 1 in 7 cells is a primitive hematopoietic stem cell, and this year in human bone marrow 28% of single

Thy1^{+}Rho^{lo}CD49f^{+} cells were reported to have long term repopulating ability (Yilmaz et al, 2006). As we move ahead in our understanding of epithelial stem cell biology, the same must be required for putative epidermal stem cell markers in epidermis. The field of hematopoiesis has reached a consensus decision to uphold an *in vivo* long-term competitive repopulation assay as the 'gold standard' for defining a stem cell (Purton and Scadden, 2007)(Chang et al, 2000). Interestingly, multipotent progenitors can be either long term repopulating cells or less primitive progenitors (Kondo et al, 1997; Akashi et al, 2000). While the need for such an assay in cutaneous biology is recognized (Strachan et al, 2008; Schneider et al, 2003; Charruyer et al, 2009)(Pouliot et al, 2005; Kaur et al, 2004), varying surrogate assays are used in practice, impeding the collaborative and effective advancement of cutaneous stem cell biology. Several of the most used surrogate assays have significant defects. For example, colony forming cells are commonly evaluated as a measure of the presence of stem cells. However, colony forming cells are definitely not synonymous with stem cells (see above). It is not clear whether long term repopulating primitive epidermal stem cells are a subset of or a different cell from the colony forming cell. Clonal analysis *in vitro* has the ability to measure self-renewal ability, but there is evidence that the most primitive stem cells may not have clonal capacity (hematopoietic stem cells with long-term bone marrow repopulating capacity have been shown to have minimal to no clonal capacity (Sutherland et al, 1990; Haylock et al, 1992; Budak et al, 2005).

Label retaining cells (in the hematopoietic system) are believed to identify one actively cycling subset of self-renewing stem cells but not the 'dormant' subset (van der Wath et al, 2009; Wilson et al, 2008). Since labeling in skin is only performed for a few days, if a similar situation exists in skin we are probably also only labeling a subset of epidermal stem cells. Thus, these surrogate assays have limitations and until we have a marker set for epidermal stem cells based on undisputed features of a stem cell (long term tissue repopulation (Strachan et al, 2008; Schneider et al, 2003; Charruyer et al, 2009)(Pouliot et al, 2005; Kaur et al, 2004) and/or self-renewal) it will be hard to define a hierarchy of progenitors and to provide a unique molecular signature for the most primitive epidermal precursor. Taking advantage of what appear to be 'generic' stem cell markers and profiling more primitive epidermal stem cells will hopefully aid and accelerate this process.

Progress in finding stem cell niches within the skin and epidermis has been steady and as more advanced techniques provide us with ever improved technology, we are moving towards understanding the locations and contributions of the different stem cell niches.

Aided by work in drosophila and in mammalian tissues we have also made steady progress in understanding the determinants of epidermal stem cell proliferation and differentiation. Although our understanding of the interactions between different regulatory pathways is limited there is extensive ongoing work in multiple laboratories.

A big challenge is to improve clinical therapy using epidermal stem cells, hair follicle derived multipotent cells and/or keratinocyte iPS cells. The new findings with iPS cells provide much hope for a rapid transition from basic science to the clinic.

Because it is a rapidly renewing tissue, similar to the gastrointestinal and hematopoietic systems, the epidermis is an attractive tissue in which to study stem cell biology. While our specialty is relatively small, keratinocytes now have the attention of the stem cell world, as an easily accessible population of cells, for many purposes. Our rapidly evolving understanding of epidermal stem cells has many potential uses that promise to translate into improved patient therapy.

REFERENCES

Aasen T, Raya A, Barrero MJ, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotech. Nov; 2008 26(11):1276–1284.

Akashi K, Traver D, Miyamoto T, et al. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature. Mar 9; 2000 404(6774):193–197. [PubMed: 10724173]

Al-Hajj M, Wicha MS, Benito-Hernandez A, et al. Prospective identification of tumorigenic breast cancer cells. Proceedings of the National Academy of Sciences. Apr; 2003 100(7):3983–3988.

Armstrong L, Stojkovic M, Dimmick I, et al. Phenotypic characterization of murine primitive hematopoietic progenitor cells isolated on basis of aldehyde dehydrogenase activity. Stem Cells (Dayton, Ohio). 2004; 22(7):1142–1151.

Artavanis-Tsakonas S, Matsuno K, Fortini ME. Notch signaling. Science (New York, N.Y.). Apr 14; 1995 268(5208):225–232.

Badiavas EV, Abedi M, Butmarc J, et al. Participation of bone marrow derived cells in cutaneous wound healing. Journal of Cellular Physiology. Aug; 2003 196(2):245–250. [PubMed: 12811816]

Barrandon Y, Green H. Three clonal types of keratinocyte with different capacities for multiplication. Proceedings of the National Academy of Sciences of the United States of America. Apr; 1987 84(8):2302–2306. [PubMed: 2436229]

Baxter MA, Wynn RF, Jowitt SN, et al. Study of telomere length reveals rapid aging of human marrow stromal cells following in vitro expansion. Stem Cells (Dayton, Ohio). 2004; 22(5):675–682.

Bergoglio V, Larcher F, Chevallier-Lagente O, et al. Safe selection of genetically manipulated human primary keratinocytes with very high growth potential using CD24. Molecular Therapy: The Journal of the American Society of Gene Therapy. Dec; 2007 15(12):2186–2193. [PubMed: 17712330]

Bickenbach JR. Identification and behavior of label-retaining cells in oral mucosa and skin. Journal of Dental Research. Aug.1981 60 Spec No C:1611–1620. [PubMed: 6943171]

Bickenbach JR, McCutecheon J, Mackenzie IC. Rate of loss of tritiated thymidine label in basal cells in mouse epithelial tissues. Cell and Tissue Kinetics. May; 1986 19(3):325–333. [PubMed: 3719664]

Bickenbach JR, Chism E. Selection and extended growth of murine epidermal stem cells in culture. Experimental Cell Research. Oct 10; 1998 244(1):184–195. [PubMed: 9770361]

Bilousova G, Chen J, Roop DR. Differentiation of mouse induced pluripotent stem cells into a multipotent keratinocyte lineage. The Journal of Investigative Dermatology. Apr; 2011 131(4): 857–864. [PubMed: 21150926]

Blanpain C, Lowry WE, Pasolli HA, et al. Canonical notch signaling functions as a commitment switch in the epidermal lineage. Genes & Development. Nov 1; 2006 20(21):3022–3035. [PubMed: 17079689]

Blanpain C, Fuchs Elaine. Epidermal homeostasis: a balancing act of stem cells in the skin. Nature Reviews. Molecular Cell Biology. Mar; 2009 10(3):207–217. [PubMed: 19209183]

Borue X, Lee S, Grove J, et al. Bone marrow-derived cells contribute to epithelial engraftment during wound healing. The American Journal of Pathology. Nov; 2004 165(5):1767–1772. [PubMed: 15509544]

Braun KM, Niemann C, Jensen UB, et al. Manipulation of stem cell proliferation and lineage commitment: visualisation of label-retaining cells in wholemounts of mouse epidermis. Development (Cambridge, England). Nov; 2003 130(21):5241–5255.

Braun KM, Watt FM. Epidermal Label-Retaining Cells: Background and Recent Applications. J Investig Dermatol Symp Proc. 2004; 9(3):196–201.

Brittan M, Braun KM, Reynolds LE, et al. Bone marrow cells engraft within the epidermis and proliferate in vivo with no evidence of cell fusion. The Journal of Pathology. Jan; 2005 205(1):1–13. [PubMed: 15546160]

Budak MT, Alpdogan OS, Zhou M, et al. Ocular surface epithelia contain ABCG2-dependent side population cells exhibiting features associated with stem cells. Journal of Cell Science. Apr 15; 2005 118(Pt 8):1715–1724. [PubMed: 15811951]

Carey BW, Markoulaki S, Hanna J, et al. Reprogramming of murine and human somatic cells using a single polycistronic vector. Proceedings of the National Academy of Sciences. Jan 6; 2009 106(1): 157–162.

Chang H, Jensen LA, Quesenberry P, et al. Standardization of hematopoietic stem cell assays: a summary of a workshop and working group meeting sponsored by the National Heart, Lung, and Blood Institute held at the National Institutes of Health, Bethesda, MD on September 8-9, 1998 and July 30, 1999. Experimental Hematology. Jul; 2000 28(7):743–752. [PubMed: 10907635]

Charruyer A, Ghadially R. Stem cells and tissue-engineered skin. Skin Pharmacology and Physiology. 2009; 22(2):55–62. [PubMed: 19188753]

Charruyer A, Barland CO, Yue L, et al. Transit-amplifying cell frequency and cell cycle kinetics are altered in aged epidermis. The Journal of Investigative Dermatology. Nov; 2009 129(11):2574–2583. [PubMed: 19458632]

Chen J, Astle CM, Harrison DE. Development and aging of primitive hematopoietic stem cells in BALB/cBy mice. Experimental Hematology. May; 1999 27(5):928–935. [PubMed: 10340409]

Chen Z, Evans WH, Pflugfelder SC, et al. Gap junction protein connexin 43 serves as a negative marker for a stem cell-containing population of human limbal epithelial cells. Stem Cells (Dayton, Ohio). May; 2006 24(5):1265–1273.

Cheung C, Smith CK, Hoog JO, et al. Expression and localization of human alcohol and aldehyde dehydrogenase enzymes in skin. Biochemical and Biophysical Research Communications. Jul 22; 1999 261(1):100–107. [PubMed: 10405330]

Clayton E, Doupé DP, Klein AM, et al. A single type of progenitor cell maintains normal epidermis. Nature. Mar 8; 2007 446(7132):185–189. [PubMed: 17330052]

Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell. Jun 29; 1990 61(7): 1329–1337. [PubMed: 2364430]

Cotsarelis G. Gene expression profiling gets to the root of human hair follicle stem cells. The Journal of Clinical Investigation. Jan; 2006a 116(1):19–22. [PubMed: 16395398]

Cotsarelis G. Epithelial stem cells: a folliculocentric view. The Journal of Investigative Dermatology. Jul; 2006b 126(7):1459–1468. [PubMed: 16778814]

Deng W, Han Q, Liao L, et al. Engrafted bone marrow-derived flk-(1+) mesenchymal stem cells regenerate skin tissue. Tissue Engineering. Feb; 2005 11(1-2):110–119. [PubMed: 15738666]

Dick JE. Stem cells: Self-renewal writ in blood. Nature. May 15; 2003 423(6937):231–233. [PubMed: 12748623]

Dotto GP. Notch tumor suppressor function. Oncogene. Sep 1; 2008 27(38):5115–5123. [PubMed: 18758480]

Dunnwald M, Tomanek-Chalkley A, Alexandrunas D, et al. Isolating a pure population of epidermal stem cells for use in tissue engineering. Experimental Dermatology. Feb; 2001 10(1):45–54. [PubMed: 11168579]

Ehama R, Ishimatsu-Tsuji Y, Iriyama S, et al. Hair follicle regeneration using grafted rodent and human cells. The Journal of Investigative Dermatology. Sep; 2007 127(9):2106–2115. doi: 10.1038/sj.jid.5700823. [PubMed: 17429436]

Estrach S, Cordes R, Hozumi K, et al. Role of the Notch ligand Delta1 in embryonic and adult mouse epidermis. The Journal of Investigative Dermatology. Apr; 2008 128(4):825–832. doi:10.1038/sj.jid.5701113. [PubMed: 17960184]

Fathke C, Wilson L, Hutter J, et al. Contribution of bone marrow-derived cells to skin: collagen deposition and wound repair. Stem Cells (Dayton, Ohio). 2004; 22(5):812–822. doi:10.1634/stemcells.22-5-812.

Ferrari S, Pellegrini G, Matsui T, et al. Gene therapy in combination with tissue engineering to treat epidermolysis bullosa. Expert Opinion on Biological Therapy. Apr; 2006 6(4):367–378. doi:10.1517/14712598.6.4.367. [PubMed: 16548763]

Fortunel NO, Hatzfeld JA, Rosemary PA, et al. Long-term expansion of human functional epidermal precursor cells: promotion of extensive amplification by low TGF-beta1 concentrations. Journal of Cell Science. Oct 1; 2003 116(Pt 19):4043–4052. doi:10.1242/jcs.00702. [PubMed: 12953061]

Fuchs E. The tortoise and the hair: slow-cycling cells in the stem cell race. Cell. May 29; 2009 137(5):811–819. doi:10.1016/j.cell.2009.05.002. [PubMed: 19490891]

Garza LA, Yang CC, Zhao T, et al. Bald scalp in men with androgenetic alopecia retains hair follicle stem cells but lacks CD200-rich and CD34-positive hair follicle progenitor cells. The Journal of Clinical Investigation. Feb 1; 2011 121(2):613–622. doi:10.1172/JCI44478. [PubMed: 21206086]

Ghazizadeh S, LB Taichman. Multiple classes of stem cells in cutaneous epithelium: a lineage analysis of adult mouse skin. The EMBO Journal. Mar 15; 2001 20(6):1215–1222. doi:10.1093/emboj/20.6.1215. [PubMed: 11250888]

Ghazizadeh S, Taichman LB. Organization of stem cells and their progeny in human epidermis. The Journal of Investigative Dermatology. Feb; 2005 124(2):367–372. doi:10.1111/j.0022-202X.2004.23599.x. [PubMed: 15675956]

Ginestier C, Hur MH, Charafe-Jauffret E, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell. Nov; 2007 1(5):555–567. doi:10.1016/j.stem.2007.08.014. [PubMed: 18371393]

Harris RG, Herzog EL, Bruscia EM, et al. Lack of a fusion requirement for development of bone marrow-derived epithelia. Science (New York, N.Y.). Jul 2; 2004 305(5680):90–93. doi:10.1126/science.1098925.

Harrison DE. Competitive repopulation: a new assay for long-term stem cell functional capacity. Blood. Jan; 1980 55(1):77–81. [PubMed: 6985804]

Haylock DN, To LB, Dowse TL, et al. Ex vivo expansion and maturation of peripheral blood CD34+ cells into the myeloid lineage. Blood. Sep 15; 1992 80(6):1405–1412. [PubMed: 1381625]

Hess DA, Meyerrose TE, Wirthlin L,T, et al. Functional characterization of highly purified human hematopoietic repopulating cells isolated according to aldehyde dehydrogenase activity. Blood. Sep 15; 2004 104(6):1648–1655. [PubMed: 15178579]

Hess DA, Wirthlin L, Craft TP, et al. Selection based on CD133 and high aldehyde dehydrogenase activity isolates long-term reconstituting human hematopoietic stem cells. Blood. Mar 1; 2006 107(5):2162–2169. [PubMed: 16269619]

Hodgson GS, Bradley TR. Properties of haematopoietic stem cells surviving 5-fluorouracil treatment: evidence for a pre-CFU-S cell? Nature. Oct 4; 1979 281(5730):381–382. [PubMed: 481601]

Honeycutt KA, Koster MI, Roop DR. Genes involved in stem cell fate decisions and commitment to differentiation play a role in skin disease. The Journal of Investigative Dermatology. Symposium Proceedings / the Society for Investigative Dermatology, Inc. [and] European Society for Dermatological Research. Sep; 2004 9(3):261–268.

Horvitz HR, Herskowitz I. Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question. Cell. Jan 24; 1992 68(2):237–255. [PubMed: 1733500]

Inoue K, Aoi N, Sato T, et al. Differential expression of stem-cell-associated markers in human hair follicle epithelial cells. Laboratory Investigation; a Journal of Technical Methods and Pathology. Aug; 2009 89(8):844–856.

Ito M, Liu Y, Yang Z, et al. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. Nature Medicine. Dec; 2005 11(12):1351–1354.

Itoh M, Kiuru M, Cairo MS, et al. Generation of keratinocytes from normal and recessive dystrophic epidermolysis bullosa-induced pluripotent stem cells. Proceedings of the National Academy of Sciences of the United States of America. May 24; 2011 108(21):8797–8802. [PubMed: 21555586]

Jaks V, Barker N, Kasper M, et al. Lgr5 marks cycling, yet long-lived, hair follicle stem cells. Nature Genetics. Nov; 2008 40(11):1291–1299. [PubMed: 18849992]

Jensen KB, Collins CA, Nascimento E, et al. Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. Cell Stem Cell. May 8; 2009 4(5):427–439. [PubMed: 19427292]

Jensen KB, Watt FM. Single-cell expression profiling of human epidermal stem and transit-amplifying cells: Lrig1 is a regulator of stem cell quiescence. Proceedings of the National Academy of Sciences of the United States of America. Aug 8; 2006 103(32):11958–11963. [PubMed: 16877544]

Jones PH, Harper S, Watt FM. Stem cell patterning and fate in human epidermis. Cell. Jan 13; 1995 80(1):83–93. [PubMed: 7813021]

Jones PH, Watt FM. Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. Cell. May 21; 1993 73(4):713–724. [PubMed: 8500165]

Jones PH, Simons BD, Watt FM. Sic transit gloria: farewell to the epidermal transit amplifying cell? Cell Stem Cell. Oct 11; 2007 1(4):371–381. [PubMed: 18371376]

Kaur P, Li A. Adhesive properties of human basal epidermal cells: an analysis of keratinocyte stem cells, transit amplifying cells, and postmitotic differentiating cells. The Journal of Investigative Dermatology. Mar; 2000 114(3):413–420. [PubMed: 10692098]

Kaur P. Interfollicular epidermal stem cells: identification, challenges, potential. The Journal of Investigative Dermatology. Jul; 2006 126(7):1450–1458. [PubMed: 16543901]

Kaur P, Li A, Redvers R, et al. Keratinocyte stem cell assays: an evolving science. The Journal of Investigative Dermatology. Symposium Proceedings / the Society for Investigative Dermatology, Inc. [and] European Society for Dermatological Research. Sep; 2004 9(3):238–247.

Kaur P, Potten CS. The interfollicular epidermal stem cell saga: sensationalism versus reality check. Experimental Dermatology. Sep; 2011 20(9):697–702. [PubMed: 21834906]

Knoblich JA. Mechanisms of asymmetric stem cell division. Cell. Feb 22; 2008 132(4):583–597. [PubMed: 18295577]

Kobayashi K, Rochat A, Barrandon Y. Segregation of keratinocyte colony-forming cells in the bulge of the rat vibrissa. Proceedings of the National Academy of Sciences of the United States of America. Aug 1; 1993 90(15):7391–7395. [PubMed: 8346261]

Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell. Nov 28; 1997 91(5):661–672. [PubMed: 9393859]

Koster MI. Making an epidermis. Annals of the New York Academy of Sciences. Jul.2009 1170:7–10. [PubMed: 19686098]

Koster MI, Dai D, Marinari B, et al. p63 induces key target genes required for epidermal morphogenesis. Proceedings of the National Academy of Sciences of the United States of America. Feb 27; 2007 104(9):3255–3260. [PubMed: 17360634]

Koster MI, Kim S, Roop DR. P63 deficiency: a failure of lineage commitment or stem cell maintenance? The Journal of Investigative Dermatology. Symposium Proceedings / the Society for Investigative Dermatology, Inc. [and] European Society for Dermatological Research. Nov; 2005 10(2):118–123.

Lajtha LG. Stem cell concepts. Differentiation; Research in Biological Diversity. 1979; 14(1-2):23–34.

Langton AK, Herrick SE, Headon DJ. An extended epidermal response heals cutaneous wounds in the absence of a hair follicle stem cell contribution. The Journal of Investigative Dermatology. May; 2008 128(5):1311–1318. [PubMed: 18037901]

Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. Feb 17; 1994 367(6464):645–648. [PubMed: 7509044]

Larderet G, Fortunel NO, Vaigot P, et al. Human side population keratinocytes exhibit long-term proliferative potential and a specific gene expression profile and can form a pluristratified epidermis. Stem Cells (Dayton, Ohio). Apr; 2006 24(4):965–974.

Lechler T, Fuchs E. Asymmetric cell divisions promote stratification and differentiation of mammalian skin. Nature. Sep 8; 2005 437(7056):275–280. [PubMed: 16094321]

Lemaître G, Nissan X, Baldeschi C, et al. Concise Review: Epidermal Grafting: The Case for Pluripotent Stem Cells. STEM CELLS. Jun 1; 2011 29(6):895–899. [PubMed: 21472820]

Lerner C, Harrison DE. 5-Fluorouracil spares hemopoietic stem cells responsible for long-term repopulation. Experimental Hematology. Feb; 1990 18(2):114–118. [PubMed: 2303103]

Li A, Simmons PJ, Kaur P. Identification and isolation of candidate human keratinocyte stem cells based on cell surface phenotype. Proceedings of the National Academy of Sciences of the United States of America. Mar 31; 1998 95(7):3902–3907. [PubMed: 9520465]

Li A, Pouliot N, Redvers R, et al. Extensive tissue-regenerative capacity of neonatal human keratinocyte stem cells and their progeny. The Journal of Clinical Investigation. Feb; 2004 113(3): 390–400. [PubMed: 14755336]

Lowell S, Jones P, Le Roux I, et al. Stimulation of human epidermal differentiation by delta-notch signalling at the boundaries of stem-cell clusters. Current Biology: CB. May 4; 2000 10(9):491–500. [PubMed: 10801437]

Luo B, Aster JC, Hasserjian RP, et al. Isolation and functional analysis of a cDNA for human Jagged2, a gene encoding a ligand for the Notch1 receptor. Molecular and Cellular Biology. Oct; 1997 17(10):6057–6067. [PubMed: 9315665]

Lyle S, Christofidou-Solomidou M, Liu Y, et al. The C8/144B monoclonal antibody recognizes cytokeratin 15 and defines the location of human hair follicle stem cells. Journal of Cell Science. Nov; 1998 111(Pt 21):3179–3188. [PubMed: 9763512]

Lyle S, Christofidou-Solomidou M, Liu Y, et al. Human hair follicle bulge cells are biochemically distinct and possess an epithelial stem cell phenotype. The Journal of Investigative Dermatology. Symposium Proceedings / the Society for Investigative Dermatologists, Inc. [and] European Society for Dermatological Research. Dec; 1999 4(3):296–301.

Mackenzie IC. Relationship between Mitosis and the Ordered Structure of the Stratum Corneum in Mouse Epidermis. Nature. May 16; 1970 226(5246):653–655. [PubMed: 5444930]

Mackenzie JC. Ordered Structure of the Stratum Corneum of Mammalian Skin. Nature. May 31; 1969 222(5196):881–882. doi:10.1038/222881a0. [PubMed: 4976975]

Mackenzie IC. Retroviral transduction of murine epidermal stem cells demonstrates clonal units of epidermal structure. The Journal of Investigative Dermatology. Sep; 1997 109(3):377–383. [PubMed: 9284108]

Mahjour SB, Ghaffarpasand F, Wang H. Hair Follicle Regeneration in Skin Grafts: Current Concepts and Future Perspectives. Tissue Engineering. Part B, Reviews. Sep 1.2011

Marazzi M, Crovato F, Bucco M, et al. GMP-Compliant Culture of Human Hair Follicle Cells for Encapsulation and Transplantation. Cell Transplantation. Mar 25.2011

Matic M. A subpopulation of human basal keratinocytes has a low/negative MHC class I expression. Human Immunology. Sep; 2005 66(9):962–968. [PubMed: 16360835]

Mavilio F, Pellegrini G, Ferrari S, et al. Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. Nature Medicine. Dec; 2006 12(12): 1397–1402.

Michel M, Török N, Godbout MJ, et al. Keratin 19 as a biochemical marker of skin stem cells in vivo and in vitro: keratin 19 expressing cells are differentially localized in function of anatomic sites, and their number varies with donor age and culture stage. Journal of Cell Science. May; 1996 109(Pt 5):1017–1028. [PubMed: 8743949]

Mikkola HKA, Klintman J, Yang H, et al. Haematopoietic stem cells retain long-term repopulating activity and multipotency in the absence of stem-cell leukaemia SCL/tal-1 gene. Nature. Jan 30; 2003 421(6922):547–551. [PubMed: 12540851]

Morris RJ, Fischer SM, Slaga TJ. Evidence that the centrally and peripherally located cells in the murine epidermal proliferative unit are two distinct cell populations. The Journal of Investigative Dermatology. Apr; 1985 84(4):277–281. [PubMed: 3981041]

Morris RJ, Tacker KC, Fischer SM, et al. Quantitation of primary in vitro clonogenic keratinocytes from normal adult murine epidermis, following initiation, and during promotion of epidermal tumors. Cancer Research. Nov 15; 1988 48(22):6285–6290. [PubMed: 2460219]

Morrison SJ, Weissman IL. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. Immunity. Nov; 1994 1(8):661–673. [PubMed: 7541305]

Nakamura Y, Muguruma Y, Yahata T, et al. Expression of CD90 on keratinocyte stem/progenitor cells. The British Journal of Dermatology. Jun; 2006 154(6):1062–1070. [PubMed: 16704635]

Niemann C, Watt FM. Designer skin: lineage commitment in postnatal epidermis. Trends in Cell Biology. Apr; 2002 12(4):185–192. [PubMed: 11978538]

Nijhof JGW, Braun KM, Giangreco A, et al. The cell-surface marker MTS24 identifies a novel population of follicular keratinocytes with characteristics of progenitor cells. Development (Cambridge, England). Aug; 2006 133(15):3027–3037.

Notta F, Doulatov S, Laurenti E, et al. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science (New York, N.Y.). Jul 8; 2011 333(6039):218–221.

Ohyama M, Terunuma A, Tock CL, et al. Characterization and isolation of stem cell-enriched human hair follicle bulge cells. The Journal of Clinical Investigation. Jan; 2006 116(1):249–260. [PubMed: 16395407]

Oliver RF. Whisker growth after removal of the dermal papilla and lengths of follicle in the hooded rat. Journal of Embryology and Experimental Morphology. Jun; 1966a 15(3):331–347. [PubMed: 5964281]

Oliver RF. Histological studies of whisker regeneration in the hooded rat. Journal of Embryology and Experimental Morphology. Oct; 1966b 16(2):231–244. [PubMed: 5971987]

Oshima H, Rochat A, Kedzia C, et al. Morphogenesis and renewal of hair follicles from adult multipotent stem cells. Cell. Jan 26; 2001 104(2):233–245. [PubMed: 11207364]

Pan Y, Lin MH, Tian X, et al. gamma-secretase functions through Notch signaling to maintain skin appendages but is not required for their patterning or initial morphogenesis. Developmental Cell. Nov; 2004 7(5):731–743. [PubMed: 15525534]

Park I, Qian D, Kiel M, et al. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. Nature. May 15; 2003 423(6937):302–305. [PubMed: 12714971]

Pavlovitch JH, Rizk-Rabin M, Jaffray P, et al. Characteristics of homogeneously small keratinocytes from newborn rat skin: possible epidermal stem cells. The American Journal of Physiology. Dec; 1991 261(6 Pt 1):C964–972. [PubMed: 1767823]

Pellegrini G, Dellambra E, Golisano O, et al. p63 identifies keratinocyte stem cells. Proceedings of the National Academy of Sciences of the United States of America. Mar 13; 2001 98(6):3156–3161. [PubMed: 11248048]

Pellegrini G, Ranno R, Stracuzzi G, et al. The control of epidermal stem cells (holoclones) in the treatment of massive full-thickness burns with autologous keratinocytes cultured on fibrin. Transplantation. Sep 27; 1999 68(6):868–879. [PubMed: 10515389]

Popova NV, Morris RJ. Genetic regulation of mouse stem cells: identification of two keratinocyte stem cell regulatory loci. Current Topics in Microbiology and Immunology. 2004; 280:111–137. [PubMed: 14594209]

Potten CS. The epidermal proliferative unit: the possible role of the central basal cell. Cell and Tissue Kinetics. Jan; 1974 7(1):77–88. [PubMed: 4129708]

Potten CS, Hendry JH. Letter: Clonogenic cells and stem cells in epidermis. International Journal of Radiation Biology and Related Studies in Physics, Chemistry, and Medicine. Nov; 1973 24(5): 537–540.

Potten CS, Loeffler M. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. Development (Cambridge, England). Dec; 1990 110(4):1001–1020.

Potten CS, Morris RJ. Epithelial stem cells in vivo. Journal of Cell Science. Supplement. 1988; 10:45–62. [PubMed: 3077942]

Pouliot N, Redvers RP, Ellis S, et al. Optimization of a transplant model to assess skin reconstitution from stem cell-enriched primary human keratinocyte populations. Experimental Dermatology. Jan 1; 2005 14(1):60–69. [PubMed: 15660921]

Prince ME, Sivanandan R, Kaczorowski A, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proceedings of the National

Academy of Sciences of the United States of America. Jan 16; 2007 104(3):973–978. [PubMed: 17210912]

Purton LE, Scadden DT. Limiting factors in murine hematopoietic stem cell assays. Cell Stem Cell. Sep 13; 2007 1(3):263–270. [PubMed: 18371361]

Randall TD, Lund FE, Howard MC, et al. Expression of murine CD38 defines a population of long-term reconstituting hematopoietic stem cells. Blood. May 15; 1996 87(10):4057–4067. [PubMed: 8639761]

Rangarajan A, Talora C, Okuyama R, et al. Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. The EMBO Journal. Jul 2; 2001 20(13):3427–3436. [PubMed: 11432830]

Rochat A, Kobayashi K, Barrandon Y. Location of stem cells of human hair follicles by clonal analysis. Cell. Mar 25; 1994 76(6):1063–1073. [PubMed: 8137423]

Siminovitch L, McCulloch EA, Till JE. The Distribution of Colony-Forming Cells Among Spleen Colonies. Journal of Cellular Physiology. Dec.1963 62:327–336. [PubMed: 14086156]

Santamaria P, Rodriguez-Piza I, Clemente-Casares X, et al. Turning human epidermis into pancreatic endoderm. The Review of Diabetic Studies: RDS. 2010; 7(2):158–167. [PubMed: 21060974]

Sasahara Y, Yoshikawa Y, Morinaga T, et al. Human keratinocytes derived from the bulge region of hair follicles are refractory to differentiation. International Journal of Oncology. May; 2009 34(5):1191–1199. [PubMed: 19360332]

Sauvageau M, Sauvageau G. Polycomb group proteins: multi-faceted regulators of somatic stem cells and cancer. Cell Stem Cell. Sep 3; 2010 7(3):299–313. [PubMed: 20804967]

Schieke SM, Ma M, Cao L, et al. Mitochondrial metabolism modulates differentiation and teratoma formation capacity in mouse embryonic stem cells. The Journal of Biological Chemistry. Oct 17; 2008 283(42):28506–28512. [PubMed: 18713735]

Schneider TE, Barland C, Alex AM, et al. Measuring stem cell frequency in epidermis: a quantitative in vivo functional assay for long-term repopulating cells. Proceedings of the National Academy of Sciences of the United States of America. Sep 30; 2003 100(20):11412–11417. [PubMed: 13679571]

Selver OB, Barash A, Ahmed M, et al. ABCG2-dependent dye exclusion activity and clonal potential in epithelial cells continuously growing for 1 month from limbal explants. Investigative Ophthalmology & Visual Science. Jun; 2011 52(7):4330–4337. [PubMed: 21421882]

Senoo M, Pinto F, Crum CP, et al. p63 Is essential for the proliferative potential of stem cells in stratified epithelia. Cell. May 4; 2007 129(3):523–536. [PubMed: 17482546]

Shackleton M, Vaillant F, Simpson KJ, et al. Generation of a functional mammary gland from a single stem cell. Nature. Jan 5; 2006 439(7072):84–88. [PubMed: 16397499]

Smith LG, Weissman IL, Heimfeld S. Clonal analysis of hematopoietic stem-cell differentiation in vivo. Proceedings of the National Academy of Sciences of the United States of America. Apr 1; 1991 88(7):2788–2792. [PubMed: 1672767]

Snippert HJ, Haegebarth A, Kasper M, et al. Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. Science (New York, N.Y.). Mar 12; 2010 327(5971):1385–1389.

Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science (New York, N.Y.). Jul 1.1988 241:58–62.

Spangrude GJ, Johnson GR. Resting and activated subsets of mouse multipotent hematopoietic stem cells. Proceedings of the National Academy of Sciences of the United States of America. Oct 19.1990 87:7433–7437. [PubMed: 1977160]

Strachan LR, Scalapino KJ, Lawrence HJ, et al. Rapid adhesion to collagen isolates murine keratinocytes with limited long-term repopulating ability in vivo despite high clonogenicity in vitro. Stem Cells (Dayton, Ohio). Jan; 2008 26(1):235–243.

Sudo K, Ema H, Morita Y, et al. Age-associated characteristics of murine hematopoietic stem cells. The Journal of Experimental Medicine. Sep 6.Nov 6.2000 192:1273–1280. [PubMed: 11067876]

Sutherland HJ, Lansdorp PM, Henkelman DH, et al. Functional characterization of individual human hematopoietic stem cells cultured at limiting dilution on supportive marrow stromal layers. Proceedings of the National Academy of Sciences of the United States of America. May; 1990 87(9):3584–3588. [PubMed: 2333304]

Szilvassy SJ, Humphries RK, Lansdorp PM, et al. Quantitative assay for totipotent reconstituting hematopoietic stem cells by a competitive repopulation strategy. Proceedings of the National Academy of Sciences of the United States of America. Nov; 1990 87(22):8736–8740. [PubMed: 2247442]

Tani H, Morris RJ, Kaur P. Enrichment for murine keratinocyte stem cells based on cell surface phenotype. Proceedings of the National Academy of Sciences of the United States of America. Sep 26; 2000 97(20):10960–10965. [PubMed: 11005869]

Taswell C. Limiting dilution assays for the determination of immunocompetent cell frequencies. I. Data analysis. Journal of Immunology (Baltimore, Md.: 1950). Apr; 1981 126(4):1614–1619.

Terunuma A, Jackson KL, Kapoor V, et al. Side population keratinocytes resembling bone marrow side population stem cells are distinct from label-retaining keratinocyte stem cells. The Journal of Investigative Dermatology. Nov; 2003 121(5):1095–1103. [PubMed: 14708612]

Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. 1961. Radiation Research. Feb; 2011 175(2):145–149. [PubMed: 21268707]

Till JE, McCulloch EA. A Direct Measurement of the Radiation Sensitivity of Normal Mouse Bone Marrow Cells. Radiation Research. Feb 1; 1961 14(2):213–222. [PubMed: 13776896]

Tolar J, Xia L, Riddle MJ, et al. Induced Pluripotent Stem Cells from Individuals with Recessive Dystrophic Epidermolysis Bullosa. J Invest Dermatol. Apr; 2011 131(4):848–856. [PubMed: 21124339]

Trempus CS, Morris RJ, Bortner CD, et al. Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. The Journal of Investigative Dermatology. Apr; 2003 120(4):501–511. [PubMed: 12648211]

Trevisan M, Iscove NN. Phenotypic analysis of murine long-term hemopoietic reconstituting cells quantitated competitively in vivo and comparison with more advanced colony-forming progeny. The Journal of Experimental Medicine. Jan 1; 1995 181(1):93–103. [PubMed: 7807027]

Triel C, Vestergaard ME, Bolund L, et al. Side population cells in human and mouse epidermis lack stem cell characteristics. Experimental Cell Research. Apr 15; 2004 295(1):79–90. [PubMed: 15051492]

Truong AB, Kretz M, Ridky TW, et al. p63 regulates proliferation and differentiation of developmentally mature keratinocytes. Genes & Development. Nov 15; 2006 20(22):3185–3197. [PubMed: 17114587]

Tumbar T. Defining the Epithelial Stem Cell Niche in Skin. Science. Jan; 2004 303(5656):359–363. [PubMed: 14671312]

Uitto J. Regenerative medicine for skin diseases: iPS cells to the rescue. The Journal of Investigative Dermatology. Apr; 2011 131(4):812–814. [PubMed: 21407233]

Wan H, Stone MG, Simpson C, et al. Desmosomal proteins, including desmoglein 3, serve as novel negative markers for epidermal stem cell-containing population of keratinocytes. Journal of Cell Science. Oct 15; 2003 116(Pt 20):4239–4248. [PubMed: 12953062]

Wan H, Yuan M, Simpson C, et al. Stem/progenitor cell-like properties of desmoglein 3dim cells in primary and immortalized keratinocyte lines. Stem Cells (Dayton, Ohio). May; 2007 25(5):1286–1297.

van der Wath RC, Wilson A, Laurenti E, et al. Estimating dormant and active hematopoietic stem cell kinetics through extensive modeling of bromodeoxyuridine label-retaining cell dynamics. PloS One. 2009; 4(9):e6972. [PubMed: 19771180]

Watt FM, Estrach S, Ambler CA. Epidermal Notch signalling: differentiation, cancer and adhesion. Current Opinion in Cell Biology. Apr; 2008 20(2):171–179. [PubMed: 18342499]

Weissman IL. The road ended up at stem cells. Immunological Reviews. Jul.2002 185:159–174. [PubMed: 12190929]

Weissman IL. 50 years later: remembering the paper. Radiation Research. Feb; 2011 175(2):143–144. [PubMed: 21268706]

Williams SE, Beronja S, Pasolli HA, et al. Asymmetric cell divisions promote Notch-dependent epidermal differentiation. Nature. Feb 17; 2011 470(7334):353–358. [PubMed: 21331036]

Wilson A, Laurenti E, Oser G, et al. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell. Dec 12; 2008 135(6):1118–1129. [PubMed: 19062086]

Withers HR. The dose-survival relationship for irradiation of epithelial cells of mouse skin. The British Journal of Radiology. Mar; 1967a 40(471):187–194. [PubMed: 6019041]

Withers HR. Recovery and repopulation in vivo by mouse skin epithelial cells during fractionated irradiation. Radiation Research. Oct; 1967b 32(2):227–239. [PubMed: 6051472]

Wu Y, Chen L, Scott PG, et al. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells (Dayton, Ohio). Oct; 2007 25(10):2648–2659.

Wu Y, Zhao RCH, Tredget EE. Concise review: bone marrow-derived stem/progenitor cells in cutaneous repair and regeneration. Stem Cells (Dayton, Ohio). May; 2010 28(5):905–915.

Xie T, Spradling AC. A niche maintaining germ line stem cells in the Drosophila ovary. Science (New York, N.Y.). Oct 13; 2000 290(5490):328–330.

Yamamoto N, Tanigaki K, Han H, et al. Notch/RBP-J signaling regulates epidermis/hair fate determination of hair follicular stem cells. Current Biology: CB. Feb 18; 2003 13(4):333–338. [PubMed: 12593800]

Yilmaz OH, Kiel MJ, Morrison SJ. SLAM family markers are conserved among hematopoietic stem cells from old and reconstituted mice and markedly increase their purity. Blood. Feb 1; 2006 107(3):924–930. doi:10.1182/blood-2005-05-2140. [PubMed: 16219798]

Yu Y, Flint A, Dvorin EL, et al. AC133-2, a novel isoform of human AC133 stem cell antigen. The Journal of Biological Chemistry. Jun 7; 2002 277(23):20711–20716. [PubMed: 12042327]

Van Zant G. Studies of hematopoietic stem cells spared by 5-fluorouracil. The Journal of Experimental Medicine. Mar 1; 1984 159(3):679–690. [PubMed: 6699542]

Zhou JX, Jia LW, Yang YJ, et al. Enrichment and characterization of mouse putative epidermal stem cells. Cell Biology International. 2004; 28(7):523–529. [PubMed: 15261160]

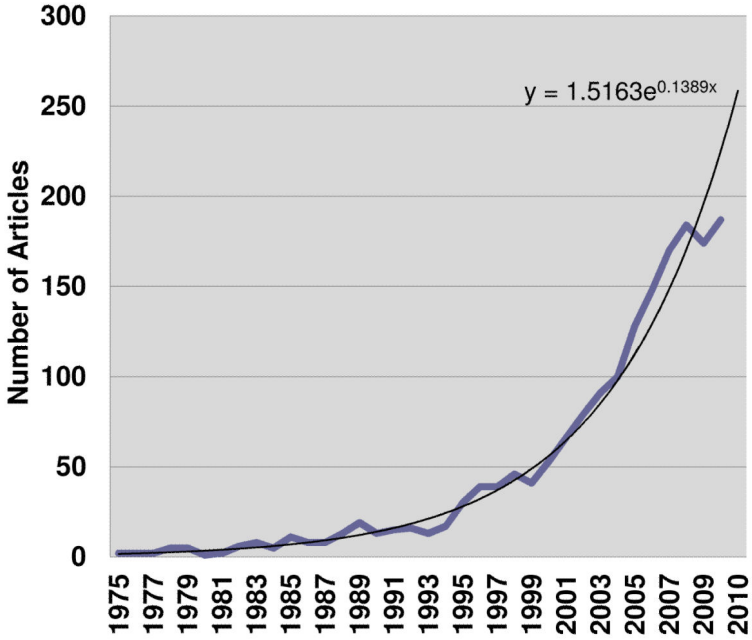

FIGURE 1.
There was an exponential increase in epidermal stem cell publications from 1985 to 2010.

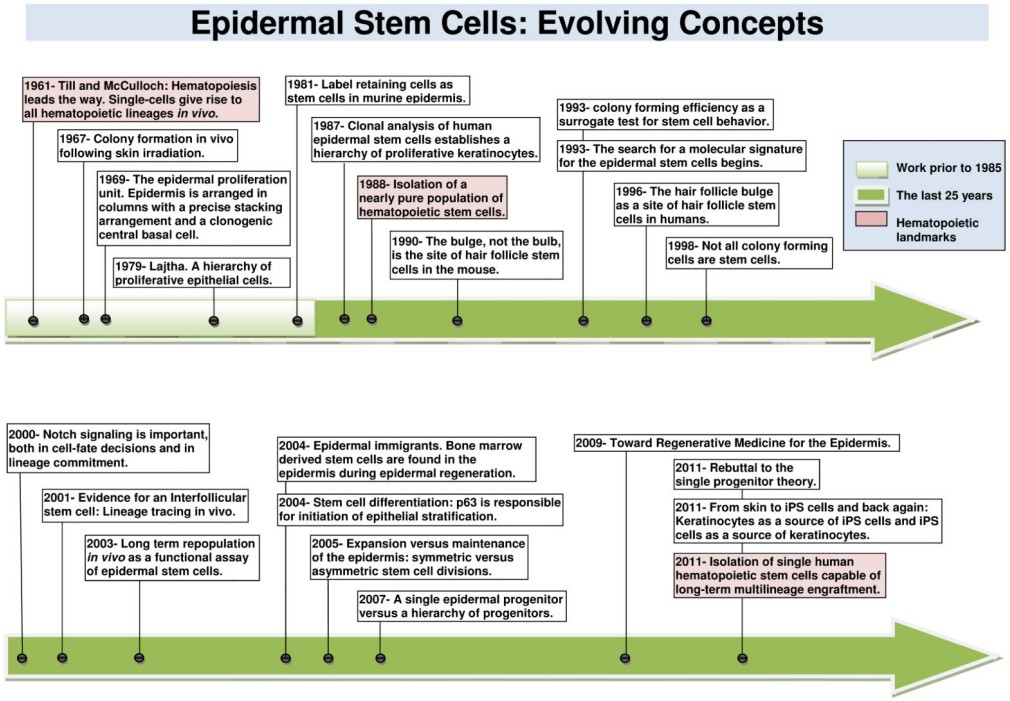

FIGURE 2.

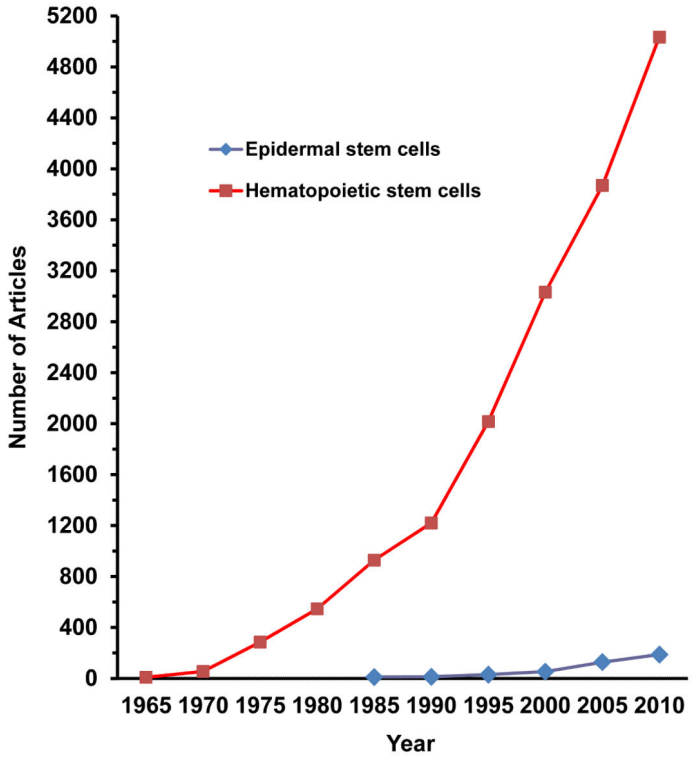

FIGURE 3.

The field of epidermal stem cell research was born 20 years after that of hematopoietic stem cell research.

Table 1

Putative Stem Cell Markers in Epidermis

MARKER (function)	STEM CELL PROPERTIES*
α6integrin^{hi} (Adhesion)	α6 integrinhi human keratinocytes were label retaining, quiescent, exhibited high nuclear to cytoplasmic ratio, high colony formation capacity and had the greatest tissue regeneration capacity (Amy Li et al. 2004; P Kaur and Li 2000).
β1 integrins (Adhesion)	Human keratinocytes that adhere rapidly to type IV collagen, a β1 integrin ligand, exhibited high proliferative potential *in vitro* (P H Jones, Harper, and Watt 1995).
BrdU/LRC (Incorporated into DNA)	Murine label retaining cells exhibited high colony forming efficiency *in vitro* and the highest integrin levels (Braun and Watt 2004).
CD133 (Prominin)	Co-expressed with β1 integrin in the basal layer of neonatal human epidermis (Yu et al. 2002).
CD200+ (Auto immunity)	Located in murine follicular bulge. High colony forming efficiency and in G0/G1 phase (Inoue et al. 2009).
Side population (SP) (Efflux Hoechst 33342)	Using a modified Hoechst 33342 technique, more than 90% of putative murine stem cells were in G0/G1 and these cells formed larger, more expandable colonies *in vitro*, than other fractions (Dunnwald et al. 2001). Human SP cells were enriched in quiescent cells, but were not label retaining cells and had low expression of surface antigens traditionally thought to mark stem cells (Terunuma et al. 2003). Murine SP cells were found to express keratin 14, β1 integrin, and p63 (J.-X. Zhou et al. 2004). Both murine and human SP cells were a subset of the α6 integrin positive cells and human SP cells expressed the drug transporter ABCG2 (Triel et al. 2004). SP cells exhibited high short- and long-term proliferative potential and formed a pluristratified epidermis (Larderet et al. 2006).
ABCG2^{+} (ATP binding cassette protein)	Human side population cells expressed the drug transporter ABCG2 (Triel et al. 2004).
Keratin 19 (Structural protein)	Co-localized with label retaining cells in mice (Michel et al. 1996).
CD34^{+} (Cell-cell adhesion factor)	Marked murine bulge keratinocytes (but not human). Predominantly in G0/G1, and expressed higher a6 integrin (Inoue et al. 2009). Refractory to differentiation in culture (Sasahara et al. 2009). Predominantly in G0/G1and co-localized with label retaining cells and keratin 15 positive cells (Trempus et al. 2003).
Aldehyde dehydrogenase (ALDH+) (Cytosolic enzyme for biotransformation of alcohols and aldehydes)	Expressed in human epidermis (Cheung et al. 1999) (150). Marker of hematopoietic (Armstrong et al. 2004; Hess et al. 2004; Hess et al. 2006) and mammary stem cells (Ginestier et al. 2007).
CD44^{+} (hyaluronic acid receptor)	Marker of mammary stem cells (Al-Hajj et al. 2003) and head and neck squamous cell cancer stem cells (Prince et al. 2007).
CD90^{+} (156) anchored cell surface protein)	Human CD90^{+} cells formed larger clusters compared to CD90- cells, when injected in NODSCID mice (Nakamura et al. 2006).
Membrane potential (Δ) (Voltage difference [interior and exterior of mitochondria])	Δ^{lo} murine embryonic stem cells possessed enhanced differentiation capacity compared to Δ^{hi} cells (Schieke et al. 2008).
Lgr5 (leucine-rich G protein-coupled receptor)	Lgr5+ murine keratinocytes were actively proliferating and multipotent stem cells able to give rise to new hair follicles for the long-term (Jaks et al. 2008)
Lgr6 (leucine-rich G protein-coupled receptor)	Adult Lgr6+ murine keratinocytes were capable of long-term wound repair including the formation of new hair follicles (Snippert et al. 2010).
MTS24 (glycoprotein)	MTS24+ murine cells expressed a6 integrin and keratin 14 and exhibited a two-fold increase in colony formation and colony size compared to MTS24-cells (Nijhof et al. 2006).
Lgr1 (leucine-rich G protein-	In murine epidermis Lgr1+ cells gave rise to all of the adult epidermal lineages in skin reconstitution assays (K. B. Jensen et al. 2009). In human epidermis Lgr1 was a regulator of

MARKER (function)	STEM CELL PROPERTIES*
coupled receptor)	stem cell quiescence (K. B. Jensen and Watt 2006).
Delta1 (Notch ligand)	Delta1 expression was confined to the basal layer of human epidermis with highest expression where stem cells reside (Lowell et al. 2000). Deletion of Delta1 under the control of keratin 5 promoter in mice, resulted in delay of the first anagen (Estrach et al. 2008).
p63 (p53 homologue)	Holoclones formed using human keratinocytes (*in vitro* clones that show less than 5% terminal colonies) showed high expression of p63 (G Pellegrini et al. 2001).
$EGFR^{lo}$ (Epidermal growth factor receptor)	Human $EGFR^{lo}$ cells generated a pluristratified epidermis in a model of skin reconstruction after long-term expansion (Fortunel et al. 2003).
$CD24^{lo}$ (glycoprotein)	$CD24^{-}$ was a more primitive mammary cell than $CD24^{lo}$ or $CD24^{hi}$. CD24 is a marker of postmitotic human keratinocytes (Bergoglio et al. 2007).
(MHC) Class I-$HLA^{low/negative}$ (Self-nonself discrimination)	Low/negative expression in a subpopulation of basal human keratinocytes (Matic 2005). Embryonic stem cells lack MHC class I antigens.
Connexin43 (Cx43dim) (Gap junction protein)	10% of human basal keratinocytes were Cx43 negative, as determined by flow cytometry. The cells were small and low in granularity, and most murine label-retaining cells did not express Cx43 (Z. Chen et al. 2006). $Cx43^{dim}$ human limbal epithelial cells are small cells, low granularity, contain high percentage of LRCs, and are positive for p63, ABCG2, and integrin β1 (Z. Chen et al. 2006).
Desmoglein3 ($Dsg3^{dim}$) (Intercellular junction protein)	High β1 integrin-expressing human keratinocytes had low levels of Dsg3. $Dsg3^{dim}$ keratinocytes had greater long-term proliferative capacity *in vitro* than $Dsg3^{bri}$. Primary adult $Dsg3^{dim}$ cells showed comparable clonogenicity to $\alpha6^{hi}CD71^{lo}$ cells (Wan et al. 2003; Wan et al. 2007).
$CD71^{lo}$ (Transferrin receptor)	Human $\alpha6$ $integrin^{hi}$ $CD71^{hi}$ keratinocytes have the greatest tissue regeneration capacity (Li, Simmons, and Kaur 1998).
CD146 MCAM (Probable adhesion molecule)	Using multiple markers along with $CD146^{lo}$, selected for human hair follicle cells with high colony forming efficiency (Ohyama et al. 2006).

Table 2

Evidence for an interfollicular stem cell (2001-present)

Reference	Conclusion/ Interpretation	Study Aim	Technical approach	Result
Ghazizadeh & Taichman, 2001	Existence of multiple stem cells with restricted lineages in cutaneous epithelium	To determine the distribution of stem cells and their progeny in epidermis	*In situ* retroviral mediated gene transfer to mark cutaneous epidermal stem cells (*lacZ* reporter gene)	Staining pattern consistent with multiple stem cell pools
Braun, 2003	Interfollicular epidermis is maintained by a stem cell compartment distinct from the hair follicle stem cell compartment	To determine the number and location of label retaining cells in the epidermis	Observation of label retaining cells in whole-mounts using histological analysis and immunolabeling of tissue sections	Label retaining cells frequently observed as individual, non clustered interfollicular cells (Ki67 negative)
Ito, 2005	Follicular stem cells do not contribute to epidermis under homeostatic conditions, but are recruited after injury	To determine whether bulge cells are required for epidermal homeostasis and repair	Ablation of bulge cells by a suicide gene encoding herpes simplex virus thymidine kinase using the keratin 15 promoter and lineage mapping	Ablation of hair follicle stem cells leads to complete loss of hair follicles but survival of the epidermis
Levy, 2005 (38) Levy, 2007	The interfollicular stem cell population is distinct from the follicular stem cell population	To determine whether stem cells of the epidermis derive from the follicle during homeostasis (2005) and wound healing (2007)	Lineage analysis of sonic hedgehog gene (ShhGFPcre; R26R)-expressing follicular cells	No labeled bulge derived cells were observed in the epidermis (2005). Follicular cells participate in initial wound resurfacing and their progeny persist in the wound for months
Langton, 2008	Hair follicle stem cells are important but not necessary for normal interfollicular epidermis homeostasis or wound healing	To determine the role of hair follicle stem-derived cells in wound healing	Wound healing analysis of Edaradd mutant mouse (developmental defects in hair follicle formation)	Hair follicle-derived stem cells participate in acute wound closure. In the absence of hair follicles a larger area of interfollicular epidermis is recruited

Adapted from Charruyer and Ghadially, What's New in Dermatology, 2011.