REVIEW ARTICLE

Hair Follicle Stem Cells: Molecular Basis of Development and Regeneration

Shirin Farivar[1, 2*], Mahboobeh RojhanNejad[1]
1. Department of Genetics, Faculty of Biological Science, Shahid Beheshti University (GC), Tehran, Iran
2. Laser and Plasma Research Institute, Shahid Beheshti University (GC), Tehran, Iran

ABSTRACT

One of the accepted models for tissue homeostasis is the capability of stem cells for self-renewal with the aid of asymmetric cell division which can give rise to a new stem cell and a daughter progenitor cell. Hair follicle can reconstitute itself in a regulated cyclical program, which suggests the presence of stem cells in it. Hair follicle morphogenesis takes place in epidermis developmental stage and strongly depends on ectoderm-mesoderm interactions and regulated signalling pathways such as Wnt, Notch, Sonic Hedgehog and Bmp. Also recent investigations have proved heterogeneity of follicle stem cells and their dynamic nature in growth cycles. Studies show that bulge cells have high proliferation potential and multipotency, and are capable to regenerate hair follicle, epidermis, and sebaceous gland as well. Moreover, identification of cell surface markers helped researchers to isolate enriched bulge cell populations from human and mouse follicles. Future treatments for follicle disorders and potential gene therapy applications in other related diseases such as tumours originating from follicle stem cells would be possible using these specific markers.

Keywords: Hair Follicle, Bulge, Multipotency, Tissue Homeostasis, Stem Cells, Self-Renewal

The adult hair follicle posses' stem cells and has cyclical growth and governs differentiation of multiple cell types and finally produce a pigmented hair shaft. These stem cells are said to be located in an area called bulge (1, 2). Hair follicles are regenerated in programmed phases of growth including anagen, catagen, and telogen. Like most other somatic stem cells, hair follicle stem cells (HFSCs) have the capacity for self-renewal with the potential to regenerate all epithelial lineages of hair follicle through life time(3, 4). The capability to renew this complex organ has made HFSCs an interesting model for studying mechanisms regulating stem cell maintenance, growth and differentiation (3, 4). Here we intend to review the biology of hair follicle (HF), HF morphogenesis, its regulating molecular mechanism, its stem cell types and their differentiation capacity (5).

Anatomy of hair follicle

From the anatomical view, HF has three compartments: infundibulum, isthmus, and the lower

* **Correspondence**:
Email: s_farivar@sbu.ac.ir

Open Access Licensed as **CC-BY**

part. The upper part is permanent while the lower part is regenerated in each growth cycle.

Isthmus is the middle permanent part; the lower part is composed of suprabulbar area and the hair bulb surrounding dermal papilla. The epithelial part of HF is isolated from the surrounding dermis with a connective tissue or dermal sheath which consists of inner basement membrane and outer connective tissue sheath. Major parts of follicle from innermost to the outermost area includes: hair shaft (HS), inner root sheath (IRS), outer root sheath (ORS), and the connective tissue sheath (CTS) as shown in Figure 1.

Dermal papilla: papilla is one of the most important governors which help the follicular structure to grow and produce a pigmented hair shaft. It is also a significant source of paracrine factors necessary for hair growth and melanisation. Some of these factors are: noggin to induce hair growth, keratinocyte growth factor and its receptor fibroblast growth factor receptor 2, hepatocyte growth factor for hair development, insulin like growth factor-1 important for morphogenesis and stem cell factors for proliferation, differentiation and melanin production (6).

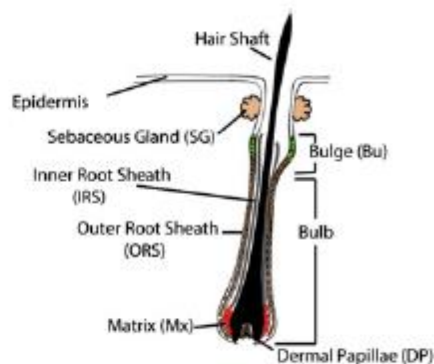

Figure 1. Anatomical view of hair follicle (7).

Hair follicle morphogenesis and regulating molecular mechanism

Formation of HF in the embryo needs interactions between surface epithelium cells and the dermis. Induction, organogenesis and cyto-differentiation are classified as three stages of morphogenesis. In the first stage, HF progenitors that called placodes are produced in competing interaction of repressors and inducers. Wnt/B-catenin signaling can turn on hair follicle fate (8). Thickening of epithelial cells to form placode is mediated through WNT signaling. There are also lots of other inducing molecules such as FGFs expressed in surface epithelium that gradually accumulate in placode region. For early placode formation, Tabby/Down less ligand receptor is necessary. SHH and its receptor Patched-1 are also expressed in epithelial placodes and underlying mesenchymal condensations. Interestingly, different hair types require different signals for placode formation. For example, primary guard hair follicle growth needs stimulation of Eda-A1/Edar/NF-kB which then up-regulates shh and cyclin D1 expression followed by placode growth. In the second stage, dermal cells underlying the epithelial cells proliferate and form a dermal condensate. Then this dermal condensate signals the epithelial cells to grow downward into the dermis. In other words, a complex interplay of signals (mostly WNT proteins) from placode to the lower dermis induces condensation of a cell population which in the next stage becomes enveloped with follicular epithelial cells to form dermal papilla (DP). In β-catenin mutants, no placode formation is found and bmp and shh are not expressed as well which indicate that these are downstream events. Moreover, ectopic expression of Wnt inhibitors such as Dkk1 causes lack of follicle induction stage. Platelet derived growth factor A (PDGF-A) is also needed for DP formation. It has been reported that complete hair loss in K14-Cre/floxed β-catenin mice is observed 4 weeks after birth and initial growth of hair, which is after the end of the first anagen phase.

The second dermal signal from accumulated region to the follicular epithelium leading to directed proliferation of follicular epithelial cells toward dermis also needs SHH expression in the epithelium (9). Generation of epidermal neoplasia and alteration in HF formation is observed in deregulations of shh signalling (10). IRS formation and HS differentiation is under control of reciprocal signals from different follicular epithelial cells (9). Notch signaling also has a significant role in follicle development, follicular fate selection of adult bulge stem cells and maintaining follicular structure (11). Another pathway that plays a role in morphogenesis is BMP signaling. Enhanced activation of BMP signals like BMP4 or inactivation of extracellular BMP inhibitor noggin leads to retardation of HF induction and baldness. In the embryo expression of

Farivar and RojhanNejad, 2015. Journal of Genes & Cells, 1(3): p, 57-66
doi: 10.15562/gnc.20

noggin by mesenchyme induces follicle morphogenesis and also new follicle growth postnatal. After initiation, embryonic follicles express BMP4 to prevent production of another HF nearby as a negative feedback (12, 13). WNT, BMP and NOTCH signaling pathways and LEF1, FOXN1, HOXC13 transcription factors are necessary for regulating follicle morphogenesis and differentiation

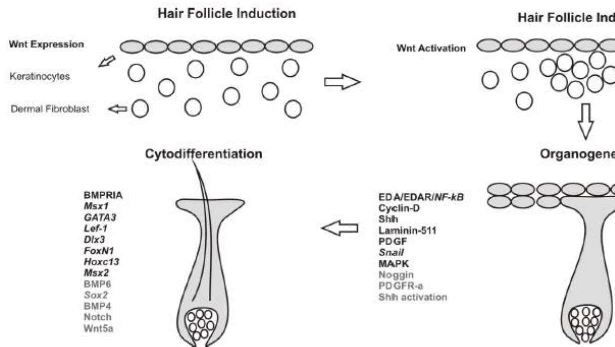

Figure 2. Hair follicle morphogenesis and required signals (14).

Hair follicle growth cycles and regulating molecular mechanism

Organogenesis of most organs occurs only once in embryogenesis, however, it differs for hair follicles. For generation of new hair, present follicles should undergo cyclical growth phases to produce HS from tip to root. In catagen and telogen, follicles reset again and prepare their stem cells to get the next anagen signals (15).

When a new follicle in each cycle is produced, it may be similar to the previous hair or differs like summer brown hair and winter white hair of Scottish bears. The type of HF is mostly dependant to regulating dermal papilla (16).

Anagen: from histological view, anagen follicles are tall (long) and flat. Proliferating matrix cells has 18 hour cycle. These cells form a new hair bulb below bulge in hair germ compartment. Progeny cells migrate to the upper parts and contribute to one of 6 lineages of IRS and hair shaft. Finally HS cells fully differentiate and their organelles exit and become packaged in associate with keratinized filaments rich in cysteine (7). Each anagen is accompanied with angiogenesis that most possibly originated from CTS (17). Anagen determines the length of the hair shaft by means of inner root sheath (IRS) and hair shaft (14). There is also another anagen dependant phenomenon, which is called follicular melanogenesis. Follicular melanogenesis ends with down regulation of its key enzymes early in anagen catagen transition (8).

Factors stimulating bulge cells for anagen onset seem to be from DP origin. FGF7 expressed in DP has strong mitogenic effects on epithelial cells. In addition, suppression of TGF-β and activation of kit ligands by NOTCH signaling ensures proliferation of matrix cells in the first anagen (18). If β-catenin was removed after the first cycle, the hair follicle is completely lost. Also in β-catenin null mutations, stem cells are directed to epidermal fate rather than keratinocytes (9).

Anagen to catagen transition: When the number of matrix cells, also called transitory amplifying cells, is reduced, HS and IRS differentiation slows down and after that follicle enters a regression phase known as catagen. Some of molecular regulators in this transition phase are: FGF5, EGF, neurotrophins like BDNF and TGF-B family members such as TGF-B1 and BMPR1a.

Catagen: This phase includes apoptosis of epithelial cells in bulb region and ORS. Hair shaft differentiation stops in catagen and its ending forms a globular structure called the club hair, which goes up to the permanent part and stays there during telogen. Production of a temporary layer known as epithelial layer is restricted to catagen which links DP to the upper part and possess dead cells. When DP reaches to the cells surrounding the club hair, this layer is removed completely. Apoptosis is an important feature of catagen follicle; different follicle compartments and cell population demonstrate distinct apoptosis capacities. For instance, DP fibroblasts and some of melanocytes that are meant to survive, show high resistance while lots of epithelial cells and melanocytes are sensitive to apoptosis. One important molecule in apoptosis progression is P53. Mutant mice analysis also identified a role for TGFB1 and P75 (a neurotrophins receptor) for catagen progress. Moreover, in mice lacking hairless protein, DP cannot move upward which leads to loss of connection between bulge and DP and cease of growth cycles.

Telogen: Telogen is the resting phase. In mice the first telogen is short and lasts for 1 or 2 days, however the second one starts on 42th day of postnatal life and lasts more than 2 weeks (4, 15).

Telogen to anagen transition: During telogen estrogen receptors are expressed strongly, thus binding of 17-β-estradiol to them prevents HFs from exiting telogen phase and entering next growth phase. During this transition, expression of β-catenin and Lef1/β-catenin is detected at bulge base, where new follicle emerges anagen phase (19).

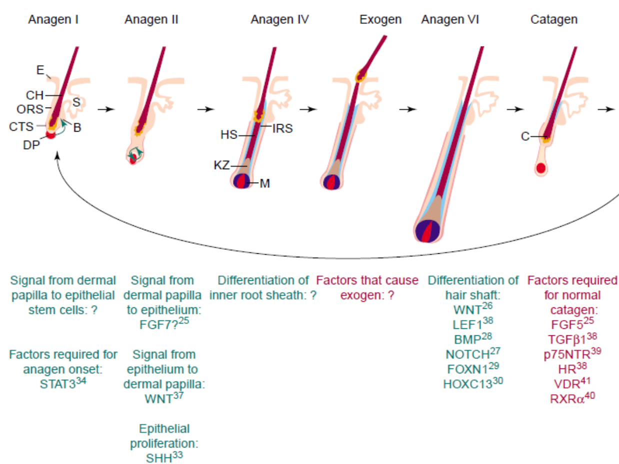

Figure 3. Factors regulating hair growth and control of the hair follicle cycle (20).

Hair follicle stem cells

Previously, it was believed that HFSCs are located in the bulb area, however it was demonstrated that after isolation of bulb which consists of matrix cells, regeneration of hair follicle was possible. Therefore, the exact area consisting of stem cells had to be identified (21-23). There are several types of stem cell populations in a hair follicle including mesenchymal stem cells, melanocyte stem cells, Nestin positive stem cells and epithelial stem cells (Figure 4).

Cotsarelis et al. found a slow cycling cell population just below the sebaceous gland and insertion site of arrectorpilli muscle known as bulge. Bulge region is in the lowest part of permanent portion of HF (21, 24).

Hair follicle mesenchymal stem cells (HFMSC) are located in CTS. Jahoda et al. demonstrated that DP and CTS primary cultures can differentiate to adipogenic and osteogenic lineages which was an evidence for their mesenchymal origin. There is also a hypothesis that HFMSCs may play a role during wound healing in dermal repair (25).

Nestin positive stem cell presence in DP was shown by Fernandes et al for the first time. After that Sieber Blum et al have located these cells in bulge region to follicle matrix. Furthermore, HF melanocyte stem cells (HF-MeSCs) have been positioned in bulge region and secondary germ in mice. This population is the reason for pigmented hair shaft produced in each cycle. Both Nestin positive stem cells and MeSCs are whether neural crest or neural crest derived stem cells that makes them beneficial for tissue engineering. Determining the origin of cells has a key role in understanding their differentiation potential. In 2004, Sieber Blum et al, found neural crest derived markers in hair follicle bulge explants cultures. This origin explains multipotency of bulge cells. Since neural crest cells migrate to different tissues including PNS and also non neural tissues during development, their derivatives have the potential to regenerate those tissues (26).

For thorough understanding of bulge biology, precise ways for bulge isolation are essential. Previously micro dissection of this region based on morphology was a common method. However, it needs expertise and also the purity of isolated population is not ensured. Therefore determining specific markers for bulge stem cells helps for a more advanced isolation and easier analysis (27-29). lyle et al, first reported that Keratin 15 is preferably expressed in human bulge region but low expression of K15 in lower portion of follicles makes it an insufficient marker for bulge (28, 30). Expression of CD34 in murine bulge cells was first reported by Trempus et al, however this surface protein does not express in human bulge cells so it cannot be considered as human bulge marker (31, 32). Microarray analysis was done for the expression profile of bulge cell population and identification of distinctly expressed genes in bulge. With the aid of microarray, a panel of human bulge surface markers was determined. CD200 and CD59 are up regulated in human bulge while CD34, CD146, CD24 are down regulated. CD200 was preferentially expressed in bulge area of human and became the best bulge surface marker. 57 Up regulated genes in murine

bulge have been identified which are of relevance to growth arrest and differentiation such as Gas1 (growth arrest specific 1), idb2 and 4 (inhibitor of DNA binding), S100 genes, Bsn and Tenacin C (Tnc), however genes dependant to mitosis, growth cycles and proliferation are down regulated (33). Also Wnt pathway activator genes are repressed in murine bulge cells which is perceptible as they induce follicular fate and differentiation (8). This expression profile suggests that the directed down regulation and up regulation of genes is important for maintaining stem cell phenotype (34). Identifying the increasing number of stem cell markers, leads to determining more details about the heterogeneity of bulge cell population. This makes it clear that HFSCs niche is heterogenic and dynamic throughout life (35, 36).

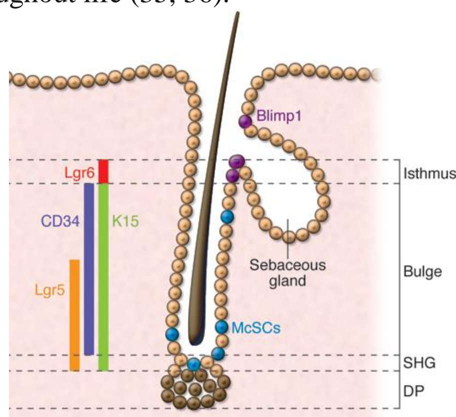

Figure 4. Different cell populations with their specific markers **(5).**

Quiescence nature of bulge cells

One salient feature of bulge cells is that they are quiescent. The use of nucleoside analogues like tritiated timidin or bromodeoxyuridin which can be taken up by cells in S phase should label slow cycling cells and once labeled they retain it for a long time detected as label-retaining cell (LRCs). In 1990, Cotsarelis et al reported that an area called bulge possess LRCs exclusively (33). In both adult mouse and human skin transplanted to immunodeficient mice tritiated timidin cannot label bulge cells except for anagen phase (37). When labeled during proliferation mouse, bulge cells can maintain label for 14 month (almost a mouse lifetime) and human bulge cells at least for 4 month (30). Surprisingly, loss of BMP signals alone is sufficient for disrupting the quiescence nature of stem cell niche. Some of the genes characteristic of bulge stem cells are SOX9, SOX4, LHX2, shh and low level of c-myc that are expanded following inhibiting BMP signalling (4, 9). Inversely, when stem cells are activated in telogen-anagen transition increased levels of c-myc and Runx1, bmp antagonists, and Wnt signaling are required (8). After identifying label retaining nature of bulge cells, the next step was to define other stemness features of these cells. Since stem cells are responsible for regeneration of their residing tissue, they have a high proliferation potential. Detecting genes that distinctly identifies bulge cells from proliferating transit amplifying cells (TA cells) and genes that turn resting bulge cells to activated ones helps researchers to understand uncontrolled proliferation nature of cancer cells and harmonic events of anagen onset as well (34). Barrandon and Green suggested that in holoclone phenotype (a stem cell feature) cells generate large colonies with smooth perimeters and low rate of terminal differentiation. It was demonstrated that holoclones were located in bulge area. Colony forming efficiency is another sign of proliferation potency which shows the ratio of colonies obtained per number of plated cells and correlates with the number of stem cells present in the cell population of interest. CFE analysis in hair follicle bulge identified colonogenic stem cells in more than 95% of cells (27). Different lineages of each tissue can be reconstituted by its stem cells. Various evidences for multipotency of bulge cells *in vivo* and *in vitro* have been achieved by different studies. Taylor et al by using a double retaining technique demonstrated that not only hair follicles but also epidermis can be produced by bulge cells (38). With transgenic labelling approaches, researchers generated fluorescent-tagged bulge cells and combined them with dermal cells for hair reconstitution assays and the result was production of hair follicle, sebaceous gland and the epidermis. Investigators also isolated bulge of Rosa26 transgenic mice that continuously express lacZ and transplanted it to a neonatal epidermis of non-Rosa mice. They observed that bulge cells could regenerate all epithelial lineages of hair follicle, sebaceous gland, and epidermis.

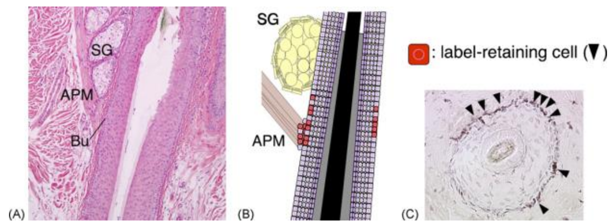

Figure 5. (A) Human hair follicle bulge is detected just as a subtle swelling. (B) LRCs are distributed in outermost layer of outer root sheath (red cells) in human hair follicle. Identification of these boundaries defined human hair follicle bulge (C) detection of LRCs in xenografted human hair follicle (arrowheads). SG: sebaceous gland; APM: arrestor pili muscle, BU: bulge (33).

Bulge cells from clinical view

not only HFSCs can produce all lineages of a follicle, but also regenerate epidermis in the site of injury.(34, 39) migration of bulge daughter cells after different injuries to the epidermis was shown by Ito et al using K15CrePR:R26R transgenic mice. In spite of bulge derived cell movement to the newly forming epidermis, the majority of them do not persist in reepithelialized epidermis which propose that bulge derived cells can contribute to healing or activate it but are not suitable for long term EPU establishment.(1) Li et al in 2003 demonstrated that in transgenic mice the regulatory element Nestin derives GFP (ND-GFP) is expressed in both neural stem cells and hair follicle stem cells.(40) moreover, Amoh et al reported that HF-ND-GFP stem cells can be converted into neurospheres in culture, which then could form neurons, glia, keratinocytes, muscle cell and etc.(41) in 2008, studies showed that transplanting HFSCs leads to better motor function following spinal cord injury.(42) furthermore, when HFSCs are injected into the region between wounded sciatic nerves, they can be regenerated. The mechanism seem to be because of HFSCs differentiation to Schwann cells.(43) HFSCs are appropriate candidates for medical purposes and even gene therapy especially because of their privilege over embryonic stem cells for ethical problems.

Alopecia areata is a common disorder of hair follicle. In this autoimmune disease, lymphocytes attack cells of anagen hair bulb. Also in a similar process after chemotherapy follicles enter dystrophic catagen and hair shaft breaks. Hair loss may occur in all scalp and body hair or just scattered patches of scalp. Potential targets for immune attack are dermal papilla, matrix cells and melanocytes. The hair loss can be reversible supporting non-scaring nature of the disease even after years of hairless due to the existence of pool of stem cells in bulge area. Another form of alopecia is Androgenetic alopecia or common baldness. In androgenetic alopecia, both male- and female-pattern, we see a decrease both in hair follicle size and duration of anagen plus an increase in telogen hair follicles. Disappearance of follicles and their replacement with fibrous tract is seen in advanced form. The For a functional treatment for alopecia, protecting follicles from immune attack or modulating inflammation is needed.(20)

Tumorgenity of bulge cells: since bulge stem cells have long lasting lifetime and slow cycling nature, they could retain mutations for long periods and become tumorgenic through time. These cells are sensitive to genetic alterations and can be a source for carcinogenic mutations. Some of hf tumors are trichofolliculoma, trichoepithelioma, trichoblastoma, trichilemmoma and trichomatricoma. There are a lot of evidences suggesting that skin tumors are derived from follicle stem cells specially bulge cells. (1, 44) over expression of sonic hedgehog genes, that are essential for hair follicle morphogenesis, can lead to formation of basal cell carcinoma.(45) evidences show that in transforming normal hf to bcc, there is an association between shh and insulin like growth factor binding protein-2 (IGFBP-2. (46) Hutchin et al in 2005 stated there is a high possibility that origin of basal cell carcinoma is within hair follicle. One evidence for this statement is the presence of bcl-2 marker for bulge cells in bcc.(47) in addition, Trichoepithelioma a hair follicle neoplasm share some common features with bcc. For example both express cytokeratins 5,6,14,17 and 19 which is the expression profile of ORS in fetal follicle bulge and germinative cells.(48, 49) moreover, there is evidence that mutation or loss of pathways such as BMP and notch could lead to some tumors, as inhibition of bmp cause trichofolliculoma in humans. (50) Determination of key molecules necessary for bulge stem cell maintenance can help researchers identify inhibitors for the growth of these tumors.(51) As it was mentioned earlier, the presence of melanocyte stem cells, Merkel cells and

langerhance cells in bulge region have been proved as well. All these data signifies the importance of bulge cells not only in keratinocytes related diseases but also in various diseases.

Hair follicle regeneration

The basis for hair regeneration is the capability of a restricted cell population of HFSCs to exit quiescence and to produce transient amplifying progeny. Anagen onset and adult HF neogenesis requires reciprocal interactions between follicular epithelial cells and adjacent mesenchymal cells in the dermis similar to embryonic follicle development.(52) studies have demonstrated a critical role for Wnt/B-catenin in initiation of follicle formation and growth as mentioned above. Wnt/b-catenin signaling is also required for wound induced hair neogenesis.(53) studies demonstrated that genetically engineered mice that express stabilized form of b-catenin display de novo HF morphogenesis.(54) modulation of pathways necessary for placode formation such as TNF family member ectodysplasin- A (EDA), SHH, and BMP (negative regulation) could lead keratinocytes to follicular fate and increase their responsiveness to dermal cell trichogenic signals. However because some epidermal tumors like basal cell carcinoma are associated with over activation of those pathways, application of this strategy needs strict regulation and monitoring. For instance, p63 plays an important role in morphogenesis and its isoform DeltaNp63 suppress follicle differentiation thus sustaining bulge cells through up regulation of sox9 is possible using siRNA interference of DeltaNp63. Also for better responsiveness to dermal signals modulation of p63 expression could be a potential strategy.(51, 55)

A lot of effort has been done for hair follicle organ transplant. In order to regenerate the follicles in growth cycles, it is necessary for stem cells and their niches to be also regenerated. Co grafting the epithelial and mesenchymal components in immunodeficient mice seems to be a functional protocol for hair follicle reconstitution. Recently some protocols like chamber assay and patch assay proved to be available for researchers. In chamber assay a silicon chamber, on which mixtures of epithelial and dermal cells are transplanted, is grafted on the back of Immunodeficient mice. In patch assay researchers inject the mixture of cells into subcutaneous space of Immunodeficient mice.(56, 57) But in experiments of epithelial-mesenchymal combination, the type of epithelial element largely affects the efficiency of reconstitution assay and phenotype of the structure, suggesting that responsiveness to dermal signals is a key factor. In those attempts that bulge cells were co-grafted with inductive dermal cells, HFs were regenerated as well as sebaceous gland and epidermis indicating that epithelial stem cells better response to inductive signals and are a desirable source for bioengineering hair follicle. (58) another strategy to prepare receptive keratinocytes was done by Ehama et al, in which dermal papilla cell population were combined with adult and neonatal keratinocytes and co-grafted in the back of nude mice. Although both cell populations formed HF-like structures that express specific markers of hair follicle lineage in primary cultures, more efficiency was observed when structures were formed by neonatal keratinocytes. Thus leading keratinocytes to embryonic state could make ameliorate strategies for better responsiveness to dermal signals. (59) In another study Fully functional follicle reconstruction was achieved through intracutaneous transplantation of bioengineered hair germ which was generated using epithelial and mesenchymal hair follicle cells of an 18E murine embryo skin. the epithelial cells were isolated from bulge and mesenchymal cells from dermal papilla.(60)

Conclusion

Hair follicle stem cells not only provide an easily accessible source compared to other stem cell sources but also have the capacity for autologous treatment as they are easily cultured and expanded in vitro after isolation. Furthermore, ethical problems of embryonic stem cells are not included in HFSc isolation, thus they could be used as an alternative source for embryonal stem cells. It is of note that, studying HFSc and signaling cascades that direct their differentiation to different lineages is very important to understand their role in some skin diseases and to developed sufficient treatments as

well. Further details on inducing signaling pathways and their reciprocal interactions in hair follicle formation could lead us to promising treatments for follicle related disorders such as alopecia. The fact that hair follicle stem cells have been proved to be multipotent in several studies suggests that they are also of great potential for regenerative medicine.

References:

1. G C. Epithelial stem cells: A folliculocentric view. J invest Dermatol. 2006;7:1459-68.
2. Paus R CG. .The biology of hair follicles. . N Engl J Med. 1999;7:491–7.
3. . Oshima H RA, Kedzia C, Kobayashi K, Barrandon. . Morphogenesis and renewal of hair follicles
from adult multipotent stem cells. Cell. 2001; . 2001;2:233–45.
4. Blanpain C LW, Geoghegan A, Polak L,. Self-renewal, multipotency, and the existence
of two cell populations within an epithelial
stem cell niche. . Cell. 2004;5:635–48.
5. Ito2 PMaM. Dissecting the bulge in hair regeneration. The Journal of Clinical Investigation. 2013;122:448-54.
6. Valerie Anne ea. The Biology of Hair Growth. Cosmetic Applications of Laser and Light-Based Systems. 2009:3-35.
7. Ying V. Zhang JC, Nichita Ciapurin, et al. Distinct Self-Renewal and Differentiation Phases in the Niche of Infrequently Dividing Hair Follicle Stem Cells. . Cell Stem Cell. 2009;5:267-78.
8. Marlon R. Schneider RS-U, et al. The Hair Follicle as a Dynamic Miniorgan. Current Biology. 2009;19.
9. Joerg Huelsken RV, et al. . b-Catenin Controls Hair Follicle Morphogenesis
and Stem Cell Differentiation in the Skin. Cell Stem Cell. 2001;105:533-45.
10. McMahon API, P.W.; Tabin, C.J. . Developmental roles and clinical significance of
hedgehog signaling. Curr Top Dev Biol. 2003;53:1-114.
11. Pan YL, M.H.; Tian, X.; Cheng, H.T et al. . Gamma-secretase functions through Notch signaling to maintain skin appendages but is not required for their
patterning or initial morphogenesis.2004, 7. Dev Cell 2004;7: 731–43.
12. Botchkarev VAB, et al. . Noggin is a mesenchymally derived
stimulator of hair-follicle induction. Nat Cell Biol. 1999;1.
13. Jamora CD, R.; Kocieniewski, et al. transcription and adhesion in epithelial bud development. . Nature 2003;422(317-322).
14. Pisal Rishikaysh KD, Daniel Diaz, et al ,. Signaling Involved in Hair Follicle Morphogenesis
and Development. Int J Mol Sci. 2014;15(1647-1670).
15. Laura Alonso EF. The hair cycle. Cell Science. 2006;119:391-3.
16. Valerie Anne ea. The Biology of Hair Growth. . Cosmetic Applications of Laser and Light-Based Systems. 2009:3-35.
17. Stephan Tiede JEK, European Journal of Cell Biology 86 (2007) 355–376. Hair follicle stem cells: Walking the maze. European Journal of Cell Biology. 2007;86:355-76.
18. . Lee GTH, J.H.; Kwak, C.; Woo, J.; et al. Effect of dominant negative transforming growth factor-beta receptor type II on cytotoxic activity of RAW 264.7,
a murine macrophage cell line. Cancer Res. 2007;67:6717-24.
19. Oh HSS, R.C. , 12525–12530. An estrogen receptor pathway regulates the telogen-anagen hair follicle
transition and influences epidermal cell proliferation. Proc Natl Acad Sci. 1996;93.
20. George Cotsarelis and Sarah E.Millar. Towards a molecular understanding of hair loss and its treatment. . TRENDS in Molecular Medicine 2001;7.
21. Cotsarelis G ST, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for
follicular stem cells, hair cycle, and skin carcinogenesis. . Cell Science. 1990;61:1329-37.
22. Akiyama M DB, Sun TT, Holbrook KA. Characterization of hair follicle bulge in human fetal skin: the human fetal

bulge is a pool of undifferentiated keratinocytes. J Invest Dermatol 1995;105:844—50.
23. Tang L MS, Lui H, Shapiro J. . Regeneration of a new hair follicle from the upper half of a human hair follicle in a
nude mouse2002;119:983—4. J Invest Dermatol 2002;119:983-4.
24. Cotsarelis G CS, Dong G, et al. Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on
epithelial stem cells. Cell Science. 1989;57:201-9.
25. Jahoda Cab RA, et al, . hair follicle dermal cells differentiate into adipogenic and osteogenic lineages. 12(6) 849 2003. Exp Detmatology. 2003;12.
26. Christine E. Wong CP, et al. Neural crest–derived cells with stem cell features can be traced back to multiple lineages in the adult skin., Vol. 175, No. 6, December 18, 2006 1005–1015. The Journal of Cell Biology. 2006;175:1005-15.
27. Kobayashi K RA, Barrandon Y. Segregation of keratinocyte colony-forming cells in the bulge of the rat vibrissae.
. Proc Natl Acad Sci USA
1993;90:7391-5.
28. Rochat A KK, Barrandon Y. Location of stem cells of human hair follicles by clonal analysis.
. Cell Science. 1994;76:1063-73.
29. Ohyama M TA, Tock CL, et al. Characterization and isolation of stem cell-enriched human hair follicle bulge cells. . J Clin Invest 2006;116:249-60.
30. Lyle S C-SM, Liu Y, et al. The C8/144B monoclonal antibody recognizes cytokeratin 15 and defines the location of human hair follicle
stem cells. 1998;111(Pt 21):3179—88. J Cell Sci 1998;111:3179-88.
31. Trempus CS MR, Bortner CD, et al. Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface
marker CD34. 2003;120:501—11. J Invest Dermatol 2003;120:501-11.
32. G. C. Gene expression profiling gets to the root
of human hair follicle stem cells. J Clin Invest 2006;116:19-22.
33. Manabu Ohyama. Hair follicle bulge: A fascinating reservoir of
epithelial stem cells. (2007) 46, 81—89. J Dermatological Science. 2007;46:81-9.
34. Morris RJ LY, Marles L, et al. Capturing and profiling adult hair follicle stem cells.
. Nat Biotechnol. 2004;22:411-7.
35. Horsley V AA, Polak L, et al. NFATc1 balances quiescence and proliferation of skin stem cells. . Cell Science. 2008;132:299-310.
36. Vidal VP ea. Sox9 is essential for outer root sheath differentiation and the formation of the hair stem cell compartment. . Curr Biol 2005;15:1340-51.
37. Ito M, Kizawa, K., Hamada, K., and Cotsarelis, G. Hair follicle stem cells in the lower bulge form the secondary germ, a biochemically distinct but functionally equivalent progenitor cell population, at the termination of catagen. Differentiation. 2004;72:548-57.
38. Taylor G LM, Jensen PJ, et al. Involvement of follicular stem cells in forming not only the follicle
but also the epidermis. . Cell Science. 2000;102:451-61.
39. Ito M LY, Yang Z, et al. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. . Nat Med 2005;11:1351-4.
40. Li L MJ, Yang M, et al. Nestin expression in hair follicle sheath progenitor cells. . Proc Natl Acad Sci USA. 2003;100:9958-61.
41. Amoh Y LL, Yang M, Moossa AR, et al. Nascent blood vessels in the skin arise from nestin-expressing hair follicle cells.
. Proc Natl Acad Sci USA. 2004;101:13291-5.
42. Ronald Sulewski aRSK. The Multipotent Nature of Hair Bulge Cells. J Investigative Dermatology. 2010;130.
43. Robert M. Hoffman. The Pluripotency of Hair Follicle Stem Cells. Cell Cycle 2006;5:232-3.
44. Jih DM LS, Elenitsas R, Cotsarelis G, et al. Cytokeratin 15 expression in trichoepitheliomas and a subset of basal cell carcinomas suggests they originate from hair
follicle stem cells. J Cutan Pathol 1999;26:113-8.
45. SE M. Molecular mechanisms regulating hair follicle development. . J Invest Dermatol 2002;118:216-25.
46. Harris PJT, N.; Ivy, S.P. Molecular conversations and the development of the hair

follicle and basal cell carcinoma. . Cancer Prev Res (Phila). 2010;3:1217-21.

47. Stenn KS LL, Veis D,. Expression of the bcl-2 protooncogene in the cycling adult mouse hair follicle.
. Journal of Investigative Dermatology. 1994;103:107-11.

48. Schirren CG BW, Sander CA, et al. Fetal and adult hair follicle: an immunohistochemical study of anticytokeratin antibodies in formalin-fixed and paraffin-
embedded tissue. . Am J Dermatopathol. 1997;19:334-40.

49. Schirren CG RA, Kaudewitz M,. Trichoblastoma and basal cell carcinoma are neoplasms with follicular differentiation sharing the same profile of cytokeratin intermediate
filaments. Am J Dermatopathol 1997;19:341-.

50. Kan LL, Y.; McGuire, T.L.; Bonaguidi, et al. Inhibition of BMP signaling in P-Cadherin positive hair progenitor cells leads to trichofolliculoma-like hair follicle neoplasias.
. J Biomed Sci. 2011:18-92.

51. Oro AE HK, Hu Z, et al. Basal cell carcinomas in mice overexpressing sonic hedgehog. . Science 1997;276:817-21.

52. S M. Molecular mechanisms regulating hair follicle development. . J invest Dermatol. 2002;118:216-25.

53. Ito M YZ, Andl T, et al. Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding. . Nature. 2007;447:316-20.

54. Gat U DR, Degenstein L, Fuchs E:. De Novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. . cell 1998;95:605-14.

55. Romano RA SK, et al. abnormal hair follicle development and altered cell fate of follicular keratinocytes in transgenic mice expressing DeltaNp63alpha. . Development 2010;137:1431-9.

56. Zheng Y DX, et al. . organogenesis from dissociated cells: generation of mature cycling hair follicles from skin-derived cells. . J Invest Dermatol. 2005;124:867-76.

57. Wienberg Wc GL, et al. . Reconstitution of hair follicle development in vivo: determination of follicle formation, hair growth, and hair quality by dermal cells. J Invest Dermatol 1993;100:229-36.

58. Manabu Ohyama OV. Strategies to enhance epithelial-mesenchymal interactions for human hair follicle bioengineering. . J of Dermatological Science. 2013.

59. Ehama R I-TY, Iriyama S, et al. Hair follicle regeneration using grafted rodent and human cells. . J Invest Dermatol. 2007;127:2106-15.

60. Koh-ei Toyoshima KA, Naoko Ishibashi, et al. . Fully functional hair follicle regeneration through the rearrangement of stem cells and their niches.
. Nature communications. 2012.