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From cell shape to cell fate via the cytoskeleton — Insights from the epidermis

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ABSTRACT

Animal cells exhibit a wide range of shapes that reflect their diverse functions. Cell shape is determined by a balance between internal and external forces and therefore involves the cytoskeleton and its associated adhesion structures. Cell shape dynamics during development and homeostasis are tightly regulated and closely coordinated with cell fate determination. Defects in cell shape are a hallmark of many pathological conditions including cancer and skin diseases. This review highlights the links between cell shape and cell fate in the epidermis, which have been studied for over 40 years both in vitro and in vivo. Briefly discussing seminal experiments showing the strong coupling between keratinocyte cell shape and their fate we primarily focus on recent studies uncovering novel cellular and molecular mechanisms linking epidermal cell shape with cell growth, differentiation, asymmetric division, and delamination.

1. Introduction

Animal cells exhibit an immense diversity in their shape and mechanics to match their wide spectrum of specialization. Ranging from flat cells, such as endothelia, through round cells, such as macrophages, to long and narrow cells, such as neurons, as well as cells with very intricate morphologies, such as photoreceptors. Each morphology is best adapted in terms of geometry, surface area, connectivity, and plasticity to execute its function within the tissue. Some adult cell types continuously and reversibly change their shape as part of their physiological function, such as muscle cells that shorten when they contract, and leukocytes that deform in order to squeeze through other tissues. In contrast, all cells undergo permanent changes in morphology during development, either as individuals or collectively within tissues, until they reach their final differentiated state. It is therefore not surprising that control of cell shape and cell fate go hand in hand [1,2]. Often, cell fate determinants are upstream of cell morphology apparatuses [3]. However, studies both in cell culture and in vivo, have shown that in many cases cell shape can also be influential upstream of cell fate, as will be discussed in this review.

2. Cell shape and the cytoskeleton

Cell shape is the manifestation of a balance of internal and external forces as well as the mechanical characteristics of the cell. The cytoskeleton, comprised of actin, microtubules, intermediate filaments, and spectrins, is responsible for force generation and for the material properties of cells and therefore is the primary system controlling cell morphology [4]. The physical nature of the cytoskeleton, and by extension of the cell, is viscoelastic [5]. At short time scales (seconds to minutes), it exhibits elastic responses to external mechanical perturbations, while on longer time scales (minutes to hours) it responds like a viscous material. The viscous response is a result of reorganization of the cytoskeletal network through polymerization, depolymerization and motor activity [6,7]. Lasting changes in cell shape are therefore possible because of the dynamic and adaptable nature of the cytoskeleton.

Underlying the cell membrane is a network of spectrin, actin, and myosin-II filaments, called the cortex, which support the membrane and maintains cell shape independent of the environment [8]. Global contraction of the cortex induces cell rounding, whereas central contraction can induce cleavage, and polar contraction can generate cortical flows that drive asymmetric protein distribution [9]. Cells can spread out and stabilize a stretched morphology by anchoring themselves at sites of cell-matrix and/or cell-cell adhesion. Such anchoring points are usually connected to arrays of actomyosin or intermediate filaments, depending on the type of adhesion receptors. Intermediate filament arrays connected at hemidesmosomes or desmosomes primarily contribute to the mechanical integrity of cells, while actomyosin networks connected at focal adhesions or adherens junctions also have an important role in mechanosensing and transmitting information on the physical state of the environment to the cell nucleus [10,11].

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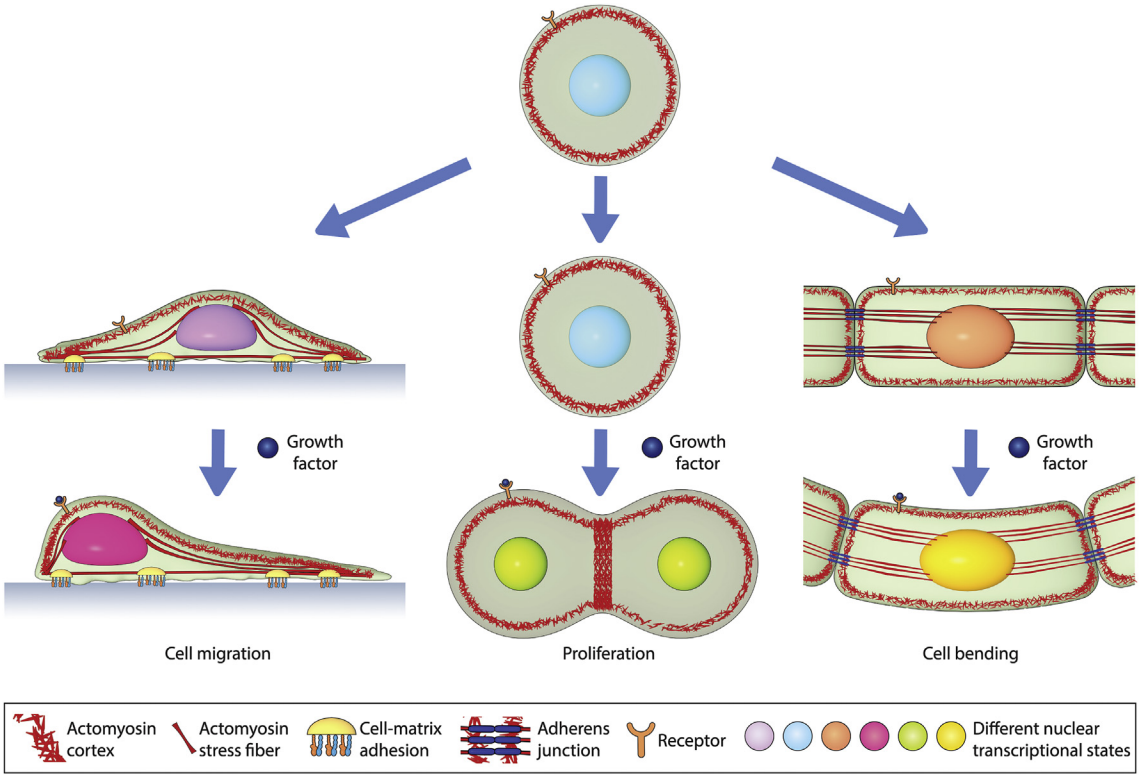


Fig. 1. Cell shape affects cell fate and vice versa, via the cytoskeleton. (A) The transcriptional state of a cell, represented by a colored nucleus, is modulated by signals from the cytoskeleton, which is influenced by the shape of the cell and its connections with the extracellular matrix and/or other cells. Non-adherent cells tend to be rounded, while adherent cells can acquire other shapes. B) Soluble growth and differentiation factors influence cell fate decisions and prompt the cell to modulate its cytoskeleton to execute a new fate. However, the same external signal will elicit different responses in different cells, depending on their shape and transcriptional state when the signal arrived. In this hypothetical example, the same growth factor induces polarized cell migration in the cell on the left, proliferation of the cell in the middle, and apical constriction of the cell on the right (leading to tissue bending).

3. Cell fate is both upstream and downstream of cell shape

Cell fate decisions integrate intrinsic factors, namely the transcriptional state of a cell, with extrinsic biochemical factors, namely growth, patterning, and differentiation signals, such as Wingless, Hedgehog, Bone Morphogenic proteins, etc. The output of a cell fate decision, be it proliferation, apoptosis, or differentiation, will activate signaling pathways that control relevant cytoskeletal dynamics. For example, in the Drosophila trachea, the trachealess patterning gene and EGF Receptor signaling activate the RhoGAP crossveinless-c that triggers cytoskeletal rearrangements, leading to epithelial invagination forming the trachea [12]. In the mouse blastocyst, internalization of the cells that will go on to form the embryo is due to an increase in their actomyosin-driven cortical tension downstream of SOX2 transcription [13,14]. However, cell shape can have an effect on the intrinsic state of the cell and therefore influence how cells respond to external signals (Fig. 1). The effect of cell shape on the transcriptional state of a cell can be mediated through specific signaling pathways, such as the Hippo pathway (leading to the transcription factors YAP/TAZ), RhoA, or serum response factor (SRF) [15–17]], or through global effects on transcription in response to forces transmitted to the nucleus [18]]. Regardless of the pathway, the end result is that a cell might respond with different fate decisions to the same external factor depending on its initial shape. For example, cytoskeletal reorganization in mesenchymal cells in pharyngeal arches of chick embryos leads to condensation, which modulates their response to growth factors; disruption of condensation inhibits differentiation of the mesenchymal cells into chondrocytes in response to fibroblast growth factor, bone morphogenic protein and transforming growth factor beta [19]. In another example, differentiation of mesenchymal stem cells into osteocytes is regulated by cell shape and actomyosin-generated tension. Restricting cell spreading and reducing cytoskeletal tension prevented BMP-induced SMAD1 translocation into the nucleus and subsequent osteogenesis [20]. Human mesenchymal stem cells that were not permitted to spread became adipocytes [21].

The notion that engineering cell shape in vitro can provide control over cell fate has motivated intense research in tissue bioengineering [22,23]. Here, we review the current knowledge on how cell shape affects cell fate in vivo, focusing on a single tissue: the skin epidermis. We start with an introduction to epidermal morphogenesis and then consider several key experiments and developmental events that exemplify how cell shape influences growth, differentiation, asymmetric division, and delamination.

4. The skin epidermis: a barrier with highly ordered cell shape and tissue architecture

The skin epidermis begins its development when cells of the surface ectoderm commit themselves to the epidermal lineage. These epidermal stem cells/progenitors will give rise to several structures, including the interfollicular epidermis (hereafter epidermis), hair follicles, sweat glands and sebaceous glands. The mature epidermis, which functions as a barrier, is a stratified epithelium that contains four main layers: i. The innermost basal layer (stratum basale) is where undifferentiated keratinocyte stem cells/progenitor adhere to the basement membrane and proliferate, ii. Spinous layer (stratum spinosum), and iii. Granular layer (stratum granulosum), in which postmitotic keratinocytes differentiate and finally, iv. A Cornified layer (stratum corneum) that contain keratinized dead cells, which are organelle and nuclei-free cells at the surface of the body [24,25].

The barrier function of the epidermis is manifested by its ability to restrict pathogens, chemicals, and radiation from entering the body and

to prevent loss of fluids from the body to the environment. Moreover, the epidermis must withstand mechanical insults and repair wounds to maintain the barrier function throughout life. To this end, the epidermis is a dynamic tissue that turns over every 2–4 weeks [26,27]. The epidermis also exhibits a robust network of keratin intermediate filaments (predominantly Keratins 5 and 14 in the basal layer and keratins 1 and 10 in suprabasal layers) and adhesion structures that maintain its structural and functional integrity [28–30].

Cell shape is another important factor that plays a crucial role in epidermal architecture and allows it to function as a barrier. During differentiation, keratinocytes undergo profound changes in cell shape. Cells in the basal layer exhibit cuboidal or short columnar architecture, whereas during differentiation they flatten. In addition to changes in cell height, cell packing geometry also differs between layers. Cells of the cornified layer are organized into columns in a honeycomb fashion. Computer simulations demonstrated that this architecture can be reached spontaneously, suggesting that the epidermis is a self-organizing system [31]. Below the cornified layer, cells of the granular layer contain tight junctions which are essential to keep fluids inside the body. These cells exhibit a flattened Kelvin's tetrakaidecahedron (f-TKD) shape which is ideal for space filling, mechanical strength and barrier function [32]. Defects in cell shape and epidermal architecture negatively affect tissue function and can be detected in common diseases such as psoriasis and cancer [33,34].

5. A link between cell shape, stemness, and mitotic potential

More than 40 years ago Howard Green and colleagues developed a method to culture keratinocytes [35–38]. In this method keratinocytes obtained from a human source are plated on feeder cells that support their survival and growth [36]. The mature colonies of keratinocytes obtained in this method have characteristics similar to the in vivo epidermis including changes in cell shape during differentiation. Small cells with growth potential are located at the basal layer while large and flat cells with cornified envelop can be detected at the most apical layers [38].

In 1985 Barrandon and Green showed that keratinocyte cell size is tightly correlated with their ability to form colonies. The authors examined cells that were isolated directly from human skin and also cultured keratinocytes. Their observations show that cells with a diameter of 11 μm or less will continue to proliferate, cells larger than 12 μm are committed to differentiation, and cells that are larger than 20 μm cannot divide [39]. Based on growth potential Barrandon and Green defined three types of keratinocyte colonies: i. Holoclones that contain stem cells and have the highest proliferative capacity, ii. Paraclones that contain transient amplifying cells and have the poorest potential, and iii. meroclones (progenitor cells) with intermediate capacity [40].

Cell size, the organization of the actin cytoskeleton and cell migration, are vastly different between the three types of colonies. At the perimeter of holoclones cells are small, whereas cells at the edge of paraclones are large [40]. Cell migration, a process that involves continuous cell shape dynamics is essential to sustain the growth of human keratinocyte colonies [41]. Moreover, a recent study demonstrated that human keratinocyte stem cells' migration can determine their stemness. A unique α6 integrin-mediated rotational movement can identify colonies with the highest growth potential already at the 2-cell colony stage [42].

In line with the crucial role of cell shape in keratinocyte stem cell growth, Nanba et al. demonstrated that the actin cytoskeleton is differentially organized in peripheral cells of holoclones and paraclones [43]. In holoclone cells, actin filaments are perpendicular to the plasma membrane whereas in paraclones actin filaments are parallel to the plasma membrane. This differential organization results in differential cell and colony shape dynamics upon activation of epidermal growth factor (EGF) signaling pathway. While holoclone colonies increase their size upon treatment, paraclone colonies shrink. The activity of the small GTPase Rac1 that plays a significant role in actin organization is essential to support both holoclone-specific cytoskeletal organization and their proliferative capacity. Rac1 inhibition in holoclones result in paraclone-like actin organization and growth potential, further emphasize the link between cell shape and growth in human keratinocytes [43]. In line with that, deletion of Rac1 depleted stem cell population also in the mouse epidermis [44]. Moreover, Rac1 activity is upregulated in keratinocytes of psoriasis patients, a disease that impacts on both cell shape and cell proliferation [44,45].

6. A link between cell shape and terminal differentiation

Fiona Watt and colleagues took a different approach to study the link between cell shape and fate. They plated a homogenous population of human keratinocyte stem cells/progenitors on adhesive islands of different sizes. This approach restricted cell contact area, and demonstrated that when the adhesive island is restricted cell proliferation decreases and the expression of involucrin, a marker of keratinocyte terminal differentiation, is upregulated [46,47]. More than 20 years later Connelly et al. took a similar experimental approach to study the mechanism that links keratinocyte shape and differentiation [48]. Similar to the original observations by Watt et al. human keratinocytes differentiated on small adhesive islands (20 μm diameter), in which keratinocytes remained rounded. In contrast, when the cells were able to spread (islands of 50 μm diameter) differentiation was less efficient. Connelly et al. demonstrated that cell size restriction alters the organization of the actin cytoskeleton. Specifically, F/G actin ratio is higher when cells are rounded than when the cells are spread. These changes activate the G-actin sensitive protein MAL that shuttles into the nucleus, binds and activates the transcription factor SRF whose target genes FOS and JUNB support keratinocytes differentiation [48]. Knockout of *Srf* in the mouse epidermis altered keratinocyte cell shape and negatively affected cell differentiation [49]. However, the complex phenotype, and particularly the severe inflammation in *Srf* conditional (c)KO epidermis make it a problematic model to understand the link between cell shape and cell fate in vivo [34,49].

Recently Totaro et al. who used a similar experimental setup, demonstrate that YAP/TAZ localization is sensitive to the shape of human keratinocyte stem cell [50]. In Large adhesive islands, YAP/TAZ localizes in the nuclei, whereas in small adhesive islands YAP/TAZ is detected predominantly in the cytoplasm. The authors show that in addition to cell shape YAP/TAZ is sensitive to a range of conditions that affect mechanical signals including substrate rigidity, cell density, and organization of the actin cytoskeleton, suggesting that a "high" mechanical forces regime is a positive regulator of YAP/TAZ activity. Interestingly, the authors found that YAP/TAZ controls keratinocytes cell fate by suppressing notch signaling. Notch signaling is a potent inducer of epidermal differentiation, and in vivo it is active in suprabasal layers [51]. The delta-like notch ligands DLL1, DLL3, and JAG2, are direct targets of YAP/TAZ, and their expression prevents keratinocyte differentiation. In vivo, overexpression of dominant active YAP induced expansion of the basal layer in which undifferentiated keratinocytes can be detected and decreased the expression of the Notch target HES1 and keratinocytes differentiation [50,52]. On the other hand, YAP/TAZ loss of function reduced the expression of DLL1 and induced premature differentiation.

Together, these results demonstrate that cell shape regulates the activity of pivotal transcription factors in stem cell/progenitor human keratinocytes: Srf and YAP/TAZ. These transcription factors control keratinocyte cell fate in vitro and in vivo.

7. Cell shape, cortical tension, and delamination

Epidermal stratification relays on two distinct mechanisms: cell delamination [53] and spindle orientation [54]. During the first stage of

epidermal stratification (E14.5-E15.5) delamination is the predominant mechanism and later on spindle orientation and asymmetric cell division becomes the predominant mechanism [55].

It is well known that keratinocyte undergoes striking cell shape changes as they move between the epidermal layers towards the surface of the body. However, recent studies documented significant cell shape changes also within the basal layer. These cell shape changes play a role in cell delamination and spindle orientation and affect gene expression and lineage commitment, as described below.

Early studies in human cultured keratinocytes demonstrated that epidermal delamination is associated with a decrease in basal cell adhesion to the basement membrane [53]. Recent studies confirmed this observation and highlighted key roles for cell shape in this process [56]. Using live cell imaging of E15.5 epidermis, Miroshnikova et al. found that the basal layer of the epidermis resembles a jammed solid-like state since cell motility, flow and neighbor exchange do not take place. Some cells in the basal layer were detected slightly above the plain of the basal layer suggesting that they are undergoing delamination. Interestingly, these cells were detected next to dividing cells. The authors suggest that cell division in the crowded basal layer generates compressive forces that alter the shape of neighboring cells. This cell shape change decreases cortical tension and increase cell-cell adhesion while decreasing its adhesion to the basement membrane and therefore promotes delamination [56] (Fig. 2).

Consistent with a key role for cell shape and cortical tension in epidermal delamination, the laboratory of Kathleen Green showed that the desmosomal cadherin, desmoglein 1 (Dsg1), is essential for epidermal stratification [57]. Dsg1 can be first detected in the epidermis as cells start to stratify where it regulates suprabasal cell shape and epidermal morphology [58]. Nekrasova et al. identified two Dsg1 binding proteins: Tctex-1, a light chain of the dynein, and cortactin, an actin-binding protein that promotes Arp2/3 mediated actin polymerization at adherens junctions. Tctex-1 is essential for the localization of Dsg1-containing desmosomes to specific cell-cell domains where it regulates cortactin- Arp2/3 dependent remodeling of the cortical cytoskeleton. This remodeling in turn will decrease cortical tension and enhance delamination [57] (Fig. 2).

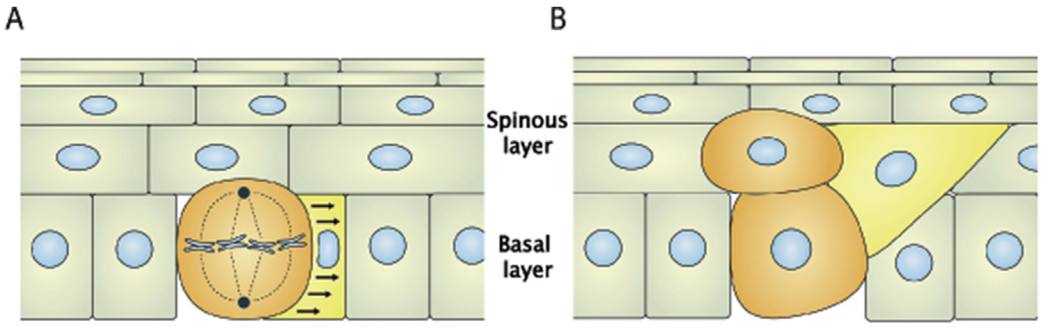
8. Cell shape, spindle orientation, and symmetric vs. asymmetric divisions

Cell division requires extensive cellular remodeling. These changes involve nuclear envelope breakdown, chromatin reorganization, redistribution of organelles and key proteins, remodeling of cell adhesions and the cytoskeleton and cell shape change. Indeed, most of the cells round-up as they enter mitosis. Failure in mitotic rounding can give rise to defects in spindle structure or spindle orientation [59–61].

As of E15.5 spindle orientation becomes the predominant mechanism for epidermal stratification [55]. When spindle orientation is parallel to the basement membrane, the two daughter cells will stay in the basal layer. However, perpendicular spindle orientation results in asymmetric cell division in which one of the daughter cells will stay in the basal layer, and the other daughter cell will move up into the suprabasal layer [62,63].

Spindle orientation and asymmetric cell division is essential for the development of epidermal barrier function [63]. In the developing hair follicle asymmetric cell division gives rise to Wnt^{Hi} and Wnt^{lo} daughter cells. Wnt^{lo} suprabasal cells will become hair follicle stem cell while the Wnt^{Hi} basal cell will become progenitor cell [64]. In skin cancer stem cells, vascular endothelial growth factor (VEGF) overexpression enhances tumor growth by promoting symmetric cell divisions of cancer stem cells and therefore increasing their number [65].

Intrinsic and extrinsic factors tightly control spindle orientation, including cell and tissue polarity, Wnt signaling, extracellular matrix (ECM), mechanical forces and cell shape [66]. In the developing mouse epidermis defect in mitotic cell shape and spindle orientation were reported when SRF [49] or WDR1 [67] were deleted. Both SRF and WDR1 are regulators of the actin cytoskeleton. SRF is a transcription factor that regulates the expression of many actin-related genes including β and γ-actin, the two fundamental building blocks of the actin cytoskeleton in keratinocytes. WDR1 enhances the actin-severing activity of cofilin and destrin. Interestingly, in the two mutants, apicobasal polarity and basement membrane organization were normal. However, the localization of LGN and NuMA that interact with apical polarity complex and align the spindle were abnormal. The mechanistic link between cell shape and spindle orientation in the developing epidermis is not clear. However, in SRF loss of function, the defects in activation of ERM (Ezrin, Radixin, and Moesin) proteins and recruitment of myosin II into the mitotic cortex may suggest a defect in the



Early changes in spindle orientation and delamination

Process: spindle orientation	Process: delamination
Cell shape changes→ mitotic rounding	Cell shape changes→ Cell area confinement
pERM ↑	Cortical tension ↓
Myosin II ↑	Basement membrane adhesion ↓
	Cell-cell adhesion ↑
	Dsg1 ↑
	cortactin–Arp2/3 mediated actin polymerization at cell junctional area ↑

Late changes in spindle orientation and delamination

Process: spindle orientation	Process: delamination
~50% of divisions (at E16.5) spindle orientation perpendicular to the basement membrane→ cell differentiation ↑	Cell differentiation ↑
~50% of divisions (at E16.5) spindle orientation parallel to the basement membrane→ cell differentiation ↓	E-cadherin mediated cell-cell junction ↑
	Cortical tension ↑

Fig. 2. Cell shape affects cell fate in the developing epidermis. The epidermis is a stratified tissue in which cells that leave the basal layer and move towards the surface of the body develop into thin and flat squamous cells. (A) As basal layer cells enter mitosis they change their shape from cuboidal/short columnar to spherical (orange cell). This cell shape change exerts compressive forces that squeeze neighboring cell (yellow cell). (B) Mitotic rounding is essential for spindle orientation that determine the position, and therefore the fate, of the daughter cells (orange cells). Defects in mitotic cell shape result in random spindle orientation. Compression forces decrease cortical tension and adhesion to the basement membrane while increasing cell-cell adhesion therefore promoting delamination (yellow cell).

mechanical properties of the cortex [68] (Fig. 2). In line with that, deletion of T-plastin (encoded by *Pls3*), a regulator of cortical actomyosin [69], in the developing epidermis leads to defect in both the cortical cytoskeleton and spindle orientation [70].

9. Cell stretching and lineage commitment

At the surface of our body, the epidermis has to withstand mechanical insults and maintain its barrier function. While the notion that the skin is a mechano-sensitive organ is well established, the molecular mechanisms that link cell stretching to cell fate decisions are poorly understood. In a recent study, the Wickstrom lab demonstrated that when keratinocytes are stretched their terminal differentiation program is inhibited [18]. Upon stretching, global repression in mRNA synthesis takes place, and the transcription of nearly 4000 genes is down-regulated. These genes are targets of the PRC2 pathway and carry H3K27me3 histone methylation.

The mechanotransduction pathway that mediates the aforementioned cell shape-cell fate coupling involves remodeling of the actin cytoskeleton and redistribution of emerin (Emd) and myosin II. Upon stretching, Emd is enriched at the outer nuclear membrane where it plays a role in the assembly of an F-actin/Myosin II rich perinuclear structure. This process increases actin polymerization in the cytoplasm and decreases the globular (G-) actin pool in the nucleus, which then impacts on global transcriptional activity that is directly coupled to H3K27me3-mediated silencing of lineage-specific genes.

In line with the involvement of Myosin II in stretch-induced repression of keratinocyte differentiation, Myh9 (encodes for Myosin IIa heavy chain) loss of function results in pre-mature differentiation during mouse epidermal development [18].

10. Concluding remarks

The skin epidermis is a tissue that exhibits a strong link between cell shape and cell fate. Early studies in cultured human keratinocytes demonstrated this behavior and recent studies continue to unravel the mechanisms that underlie this phenomenon in vitro and in vivo. Cell shape is involved in the two main mechanisms that allow the epidermis to stratify during development: delamination and spindle orientation. However, on top of “passive” regulation that involve the positioning of cells in proliferative or postmitotic environments (basal layer or spinous layer, respectively) cell shape is also linked to activation of key transcriptional factors (e.g. Srf, YAP/TAZ) and to a chromatin remodeling that control the expression of thousands of genes and determine lineage commitment.

Most (if not all) our knowledge about the role of cell shape in epidermal cell fate is limited to the interfollicular epidermis lineage, which is relatively simple. In contrast, the hair follicle is a more complex epidermal structure in which cells with different shapes, functions, and fates can be detected. One of the most interesting cell populations in the hair follicle is the hair follicle multi-potent stem cells that can be detected at the bulge. When compared to epidermal stem cells/progenitors, hair follicle stem cells exhibit a distinct cell shape [71]. In vivo studies demonstrated that a defect in hair follicle stem cell shape is correlated with their malfunction [72]. Additional work is required to determine if the mechanisms described in this review function also in other types of epidermal cells, and how these or alternative mechanisms can regulate cell fate decision in epidermal multipotent stem cells.

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