REVIEW

Crosstalk in skin: melanocytes, keratinocytes, stem cells, and melanoma

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Abstract In the vertebrate embryo, melanocytes arise from the neural crest, migrate to and colonize the basal layer within the skin and skin appendages. Post-migratory melanocytes are securely attached to the basement membrane, and their morphology, growth, adhesion, and migration are under control of neighboring keratinocytes. Melanoma is a malignant tumor originated from melanocytes or their progenitor cells. During melanocyte transformation and melanoma progression, melanocytes lose their interactions with keratinocytes, resulting in uncontrolled proliferation and invasion of the malignant cells. Melanoma cells at the advanced stages often lack melanocytic features and resemble multipotent progenitors, which are a potential melanocyte reservoir in human skin. In this mini-review, we will summarize findings on cell-cell interactions that are responsible for normal melanocyte homeostasis, stem cell self-renewal, and differentiation. Our ultimate goal is to define molecules and pathways, which are essential for normal cell-cell interactions but deregulated in melanoma formation and progression.

Keywords Melanocyte · Melanoma · Stem cell · Keratiocyte · Cell-cell interaction

Introduction

Melanoma is the deadliest form of skin cancer, with an increase in almost 80,000 new diagnoses in 2016. The disease originates from melanocytes, the pigment-forming cells of the

skin. Recent advances in cancer genomics revealed that the majority of melanoma mutations are C > T transitions and are due to UV light irradiation (Hodis et al. 2012). Risk factors for melanoma include environmental factors such as prolonged sun exposure, as well as heritable traits like fair skin, red hair, or family history. These observations suggest that UV radiation plays a role in melanomagenesis; however, the precise mechanisms underlying malignant transformation of melanocytes are still poorly understood.

The MAPK kinase pathway is activated in the vast majority of melanoma cases (van Elsas et al. 1995; Davies et al. 2002; Wan et al. 2004; Cohen et al. 2002), therefore oncogenic insults such as BRAF V600E and NRAS Q61R are considered as driver mutations for melanoma. Melanocytic nevi (moles) often harbor oncogenic mutations in BRAF and NRAS, which activate an initial phase of cell proliferation (Roh et al. 2015). Fortunately nevus growth is usually limited due to a senescence program induced by the oncogenes (Michaloglou et al. 2005). Thus, after the initial growth, most nevi remain the same size as benign moles and will not progress to melanoma. However, one half of melanomas can manifest as a benign nevus for many decades before becoming malignant. The other half arises de novo from isolated melanocytes, rather than from preexisting nevi (Goodson and Grossman 2009).

Treatment options of early-stage melanomas almost always comprises of wide resection, whereas late-stage melanomas are hard to cure with surgical treatment. Recent advances in the field have developed a barrage of pharmacological means for the late stage melanomas, including kinase inhibitors and immunotherapies (Chapman et al. 2011; Flaherty et al. 2010; Robert et al. 2014; Weber et al. 2008). Most recently, checkpoint inhibitors such as anti-PD-1 and anti-CTLA-4 antibodies represent a major breakthrough in the treatment of melanomas, promising long-term survival benefits (Hodi et al. 2010). Approximately one third of patients show durable

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responses and increased survival. However, in addition to the anti-tumor T cell responses these checkpoint inhibitors unleash, they also cause autoimmune T cell responses against healthy host tissue, resulting in significant autoimmune toxicities in many cases (Perica et al. 2015). Furthermore, the majority of melanoma cases still remains non-responsive or acquires resistance to any of the novel therapies (Johannessen et al. 2010; Nazarian et al. 2010; Zaretsky et al. 2016). Therefore, it is critical to develop approaches that prevents both initiation and progression of melanoma, as well as can overcome drug resistance.

Emerging evidence suggest that melanoma cells are more similar to progenitor cells than to differentiated melanocytes. Thus, understanding the cell-cell crosstalk responsible for normal melanocyte homeostasis and stem cell self-renewal and differentiation will help us to define molecules and pathways that become deregulated in melanoma formation and progression.

Cell-cell interactions between melanocytes and keratinocytes

The human skin is the largest organ of the body and functions to serve as the body's first line of defense against infection and to regulate temperature and fluid balance. The outermost epidermal layer is made up of a network of keratinocytes with interconnected melanocytes and scattered inflammatory cells. The dermal layer immediately below contains the fibroblasts and nerve endings, hair follicles, sweat glands, and blood vessels among other structures. The epidermis and dermis are separated and held together by the basement membrane where mature, epidermal melanocytes are anchored. Each melanocyte is in contact with 30–40 neighboring keratinocytes through long dendritic extensions that form adhesive structures (Fitzpatrick and Breathnach 1963). Melanosomes, the pigment-containing unit, are produced in melanocytes and passed on to keratinocytes where they affect skin pigmentation and protection against UV radiation (Ando et al. 2012). Consequently, the cell-cell interactions provide the opportunity for crosstalk between keratinocytes and melanocytes and for the skin microenvironment to adapt in response to external stimuli.

Various laboratory mouse strains serve a useful purpose as genetic models, however, a key difference between human and mouse skin lies in the location of melanocytes. Human melanocytes reside along the basement membrane of the epidermis, while mouse melanocytes are found in the base bulb region of hair follicles in the dermis (Nishimura et al. 2002). The change in niche gives rise to fundamental differences in skin biology and cell-cell crosstalk, as the keratinocytes of human skin control melanocytes through secretion of a variety of growth factors and cytokines in a paracrine manner. These

factors regulate growth, survival, adhesion, migration, and differentiation of melanocytes.

During development, neural crest stem cells migrate to distal parts of the embryo and differentiate to various lineages, including melanocytes of the skin. The melanocytes migrate upwards through the dermis until they come in contact with keratinocytes and securely attach to the basement membrane. Several secreted molecules were identified as melanocyte growth factors in the skin. Melanocyte division is likely initiated by keratinocyte-derived growth factors, such as basic fibroblast growth factor (bFGF), stem cell factor (SCF), or endothelin 1 (ET-1), or by fibroblast-derived factors such as bFGF, hepatocyte growth factor (HGF), or endothelin 3 (ET-3) (Haass and Herlyn 2005). FGF, SCF, and HGF bind to receptor tyrosine kinases and support human melanocyte growth in vitro synergistically through activators of the PKC pathway, such as ET-1 or ET-3 (Halaban 1994; Yaar and Gilchrist 1991). After each cell division, melanocytes separate, while securing their attachment to the basement membrane through integrins such as $\alpha 6 \beta 1$ or $\alpha 7 \beta 1$ (Haass and Herlyn 2005; Valyinyag et al. 1993).

Unlike keratinocytes, epidermal melanocytes are thought to have a very slow turnover rate in the human epidermis. Upon isolation from the skin microenvironment, melanocytes in monocultures proliferate with a doubling time every 2 to 4 days and even alter their adhesion molecule profile, closely mimicking melanoma cells. When cell-cell contact is re-established between melanocytes and undifferentiated, basal-like keratinocytes, melanocyte proliferation decreases and the expression of melanoma-associated markers such as integrin $\alpha v \beta 3$ and MelCAM down-regulate (Valyi-Nagy et al. 1993; Shih et al. 1994). These findings suggest that undifferentiated, basal keratinocytes control growth and cell surface receptor expression of melanocytes through direct cell-cell contact.

In addition to direct cell-cell contact, a normal melanocyte's phenotype is also maintained through paracrine signals that originate in the keratinocyte. Upon UV irradiation, keratinocytes secrete melanocyte growth promoting factors such as SCF and ET-1 (Imokawa et al. 1992; Hachiya et al. 2004). UV also induces the expression of pro-inflammatory cytokines in keratinocytes such as interleukin 1 (Nasti and Timares 2012). Among the pro-inflammatory cytokines secreted by keratinocytes, IL-1 β plays a role in up-regulating melanocyte secretion of the matricellular protein CCN3 (Fukunaga-Kalabis et al. 2006). This has two biological effects on melanocytes: increased attachment to the basement membrane and growth inhibition. The increased attachment to collagen IV found in the basement membrane, but not collagen I of the dermis, in human skin reconstructs is modulated through DDR1, a tyrosine kinase receptor for several collagens. Knockdown of DDR1 in melanocytes decreases their adhesion to collagen IV and delocalizes cells from the basement membrane to the upper layers of the epidermis,

mimicking the phenotype of CCN3 depletion. While CCN3 is able to inhibit the proliferation of melanocytes, the mechanism occurs separately from its regulation of adhesion, since knock-down of DDR1 does not affect the proliferation of melanocytes in skin reconstructs.

The study in normal melanocytes suggests that CCN3 possesses gatekeeper functions to secure melanocytes' attachment to the epidermal microenvironment (Fig. 1). Although laminin-integrin $\alpha 6 \beta 1$ binding is thought of as the primary mechanism for melanocyte attachment to the basement membrane, the expression of the $\alpha 6$ -integrin subunit is down-regulated by UV irradiation (Krengel et al. 2005). Instead, in UV irradiated skin, DDR1-collagen IV binding may be critical for melanocyte positioning, since our study has shown that CCN3 is up-regulated in melanocytes in response to pro-inflammatory cues from UV-irradiated keratinocytes. It is notable that recent studies revealed a link between DDR1 and a pigmentation disorder, vitiligo. The etiology of vitiligo is still under debate, however several hypotheses have been raised including melanocyte stress and immune mechanisms (Harris 2016). Several studies showed that genetic variants of the DDR1 gene are associated with vitiligo, and the expression of DDR1 is decreased in vitiligo lesions (Silva de Castro et al. 2010; Ricard et al. 2012; Reichert-Faria et al. 2013; Elgarhy et al. 2015). This accumulating evidence suggests that deregulation of the CCN3-DDR1-collagen axis is involved in vitiligo pathogenesis.

In primary melanoma, CCN3 is still highly expressed at the epidermal-dermal junction, but decreases in dermal areas of the lesion where tumor cells are invading into surrounding tissues (Fukunaga-Kalabis et al. 2008). Overexpression of CCN3 in melanoma cells reduces their growth and invasion

in the 3D skin reconstruct model. CCN3 decreases the activity of MMPs, which is associated with increased invasiveness of melanoma cells. On the other hand, CCN3 expression is re-induced in visceral metastases (Vallacchi et al. 2008) as overexpression increases metastasis to the liver in the experimental mouse metastasis models. CCN3 increases melanoma cell adhesion to collagen I, vitronectin, and laminin, but not to collagen IV, suggesting that CCN3 promotes the adhesion of melanoma cells in a different manner from that of normal melanocytes (Fukunaga-Kalabis et al. 2006). These findings suggest that in melanoma, the biological functions of CCN3 appear to be dependent on the tumor progression stage.

Cell-cell interactions between melanocyte progenitors and keratinocytes

Melanocyte precursors arise from neural crest cells and migrate to the skin during embryonic development. In postnatal mouse skin, melanocyte progenitors are mainly localized in the bulge region of hair follicles (Nishimura et al. 2002). Upon each hair cycle, melanocyte precursors differentiate and migrate into hair bulbs to become fully matured melanocytes, determining animal hair color.

Recent studies using genetic mouse models have provided information on the interactions between melanocyte progenitors and other cell types in hair follicles, which contribute to self-renewal, division, migration, and differentiation.

For self-renewal of melanocyte stem cells, collagen XVII expressed by epithelial stem cells in the bulge region is critical (Tanimura et al. 2011), as mice lacking collagen XVII present with premature hair graying and hair loss. Forced expression

Fig. 1 Within the epidermis, melanocytes adhere to the basement membrane primarily through $\alpha 6 \beta 1$ integrin contacts with laminin. UV radiation causes skin keratinocytes to release pro-inflammatory cytokine IL-1 β , which is taken up by melanocytes. In response, melanocytes

release CCN3, and in an autocrine manner, up-regulates receptor tyrosine kinase DDR1. This increases adherence with the basement membrane through contact with collagen IV

of COL17A1 in hair follicle stem cells restores TGF- β signaling and rescues the maintenance of melanocyte stem cells, suggesting that epithelial stem cells regulate the fate of melanocytes. Epithelial stem cells also play a role in differentiation of melanocyte progenitors. The expression of endothelin-1 is induced by the activation of the canonical Wnt pathway in epithelial stem cells, and secreted endothelin-1 from epithelial stem cells promotes differentiation of melanocyte progenitors to mature melanocytes (Rabbani et al. 2011). Several canonical Wnt ligands are up-regulated in melanocyte progenitors by depilation, but more robustly in epithelial stem cells. Using a mouse model, in which melanocytes are directed to the epidermis, Yamada et al. showed that Wnt7a, one of the canonical Wnt ligands induced by depilation, is up-regulated by UVB radiation in keratinocytes and epithelial hair follicle stem cells, and it triggers the differentiation of melanocyte progenitors through β -catenin activation (Yamada et al. 2013). UVB irradiation also induced kit ligand SCF in the epidermis, which elicits migration of melanocytes to the epidermis.

In humans, melanocyte stem cells in hair follicles lose their self-renewal capacity with aging, which causes hair graying, whereas skin melanocytes sustain lifelong proliferative potential and provide pigmentation. Findings from our group suggest that an extra-follicular reservoir for melanocyte stem cells exist in the postnatal epidermis of human skin (Li et al. 2010). The dermis of human skin contains multipotent cells, which are very similar to embryonic neural crest stem cells and able to differentiate to neural crest derivatives, including

melanocytes. These neural crest stem cell-like cells (NCSC-like cells) and melanoma cells share many biological properties, such as high migratory and invasive capabilities, as well as expression of neural crest markers. NCSC-like cells are very similar to the multipotent skin-derived precursors earlier reported by Miller's group. Although skin-derived precursors differentiate to neural and mesoderm progeny, the potential of skin-derived precursors for melanocytic differentiation is not known (Toma et al. 2001, 2005; Fernandes et al. 2004). NCSC-like cells display high Notch activity, which is sufficient to dedifferentiate adult melanocytes to a multipotent progenitor state (Zabierowski et al. 2011). Blocking Notch signaling by a γ -secretase inhibitor induces robust cell death specifically in sphere-forming stem cells, but not in adherent/differentiated cells, indicating that Notch signaling is required for maintenance of NCSC-like cells (Fukunaga-Kalabis et al. 2015).

The NCSC-like cells differentiate into melanocytes when maintained in three-dimensional (3D) skin reconstructs. Skin reconstructs consist of a dermis made of collagen matrix containing NCSC-like cells and fibroblasts, and an epidermis with multilayered keratinocytes, reflecting the physiological architecture of human skin. Similar to melanocyte differentiation in hair follicles, Wnt ligands expressed by keratinocytes promote differentiation of NCSC-like cells to melanocytes in the 3D skin reconstruct model (Fig. 2). When exposed to Wnt pathway blockers such as tankyrase inhibitors or porcupine inhibitors, melanocyte differentiation from NCSC-like cells is disturbed. Wnt7a, which is induced in UV-irradiated

Fig. 2 Neural crest stem cell (NCSC)-like cells reside in a dermal niche with high Notch and low NUMB activity and contain self-renewal capability. Upon UV irradiation in 3D skin reconstructs, keratinocytes release Wnt ligands that control melanocyte homeostasis. The ligands bind to receptors on NCSC-like cells, inhibit self-renewal, and induce differentiation into melanocytes

keratinocytes, rescues melanocyte emergence in reconstructs treated with a tankyrase inhibitor. Furthermore, treatment of NCSC-like cells with Wnt7a up-regulated the expression of Numb, an endogenous Notch inhibitor, which inhibits self-renewal of NCSC-like cells and helps them to enter the differentiation pathway. Taken together, upon UV exposure, skin keratinocyte-derived Wnt signaling promotes melanocyte differentiation of multipotent stem cells in the dermis, which in sequence secrete melanosomes to protect against the harmful rays of the sun.

Conclusion

Global gene expression analyses and biological characterization of melanocytes, melanoma cells, and NCSC-like cells suggest that melanoma cells are more similar to NCSC-like cells than melanocytes, suggesting that melanoma cells have a stem cell-like phenotype. Melanoma cells could be considered as “reprogramed” melanocytes, which revert to stem cells. Normal melanocyte stem cells are able to respond to differentiation cues in the microenvironment. In contrast, most melanoma cells are refractory to differentiation signals and maintain their stem cell phenotypes, which may be responsible for their propagation, metastasis, and therapy resistance. Further studies are required to identify the mechanisms underlying stem-like phenotypes in melanoma, which is likely influenced by cell-cell interactions in the tumor microenvironment. Understanding tumor-specific cell-cell interactions may ultimately lead to the development of novel therapeutic approaches.

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