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## Recent advances on skin-resident stem/progenitor cell functions in skin regeneration, aging and cancers and novel anti-aging and cancer therapies.

Murielle Mimeault  
*University of Nebraska Medical Center*

Surinder K. Batra  
*University of Nebraska Medical Center, sbatra@unmc.edu*

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## Recent advances on skin-resident stem/progenitor cell functions in skin regeneration, aging and cancers and novel anti-aging and cancer therapies

Murielle Mimeault<sup>a, b, \*</sup>, Surinder K. Batra<sup>a, b, c</sup>

<sup>a</sup> Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA  
<sup>b</sup> Eppley Institute for Research on Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE, USA  
<sup>c</sup> Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, USA

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### Abstract

Recent advances in skin-resident adult stem/progenitor cell research have revealed that these immature and regenerative cells with a high longevity provide critical functions in maintaining skin homeostasis and repair after severe injuries along the lifespan of individuals. The establishment of the functional properties of distinct adult stem/progenitor cells found in skin epidermis and hair follicles and extrinsic signals from their niches, which are deregulated during their aging and malignant transformation, has significantly improved our understanding on the etiopathogenesis of diverse human skin disorders and cancers. Particularly, enhanced ultraviolet radiation exposure, inflammation and oxidative stress and telomere attrition during chronological aging may induce severe DNA damages and genomic instability in the skin-resident stem/progenitor cells and their progenies. These molecular events may result in the alterations in key signalling components controlling their self-renewal and/or regenerative capacities as well as the activation of tumour suppressor gene products that trigger their growth arrest and senescence or apoptotic death. The progressive decline in the regenerative functions and/or number of skin-resident adult stem/progenitor cells may cause diverse skin diseases with advancing age. Moreover, the photoaging, telomerase re-activation and occurrence of different oncogenic events in skin-resident adult stem/progenitor cells may also culminate in their malignant transformation into cancer stem/progenitor cells and skin cancer initiation and progression. Therefore, the anti-inflammatory and anti-oxidant treatments and stem cell-replacement and gene therapies as well as the molecular targeting of their malignant counterpart, skin cancer-initiating cells offer great promise to treat diverse skin disorders and cancers.

**Keywords:** skin-resident adult stem/progenitor cells • senescence • aging • skin cancer stem/progenitor cells • anti-aging therapies • cancer therapies • molecular targeting

\*Correspondence to: Murielle MIMÉAULT, Ph.D.,  
 Department of Biochemistry and Molecular Biology,  
 Eppley Institute for Research in Cancer and Allied Diseases,  
 University of Nebraska Medical Center, Omaha,

NE 68198-5870, USA.  
 Tel.: (402)559-5455  
 Fax: (402)559-6650  
 E-mail: mmimeault@unmc.edu

## Introduction

Human skin epidermis and its appendages exhibit different site-specific morphologies and very important physiological functions including the protection against environmental injury, infection and excessive dehydration, corporal temperature regulation, and hair growth maintenance [1–6]. The skin integrity and barrier function are tightly regulated by a complex network of cell–cell and cell–matrix interactions. In particular, the cellular regeneration of skin epidermis and hair follicles is maintained along lifespan of individuals by different adult stem/progenitor cell subpopulations localized within the specialized microenvironments, niches in basal layer of epidermis, sebaceous gland and hair follicle bulge region (Fig. 1) [7–20]. These small subpopulations of immature cells endowed with a high self-renewal capacity and multilineage differentiation ability contribute to replenish the different skin cell lineages, including mature and specialized keratinocytes, sebocytes or melanocytes, in homeostatic conditions or after intense skin injuries. A chronic or cumulative ultraviolet-light (UV) exposure and inflammation of skin and oxidative stress and telomere shortening in skin stem/progenitor cells and their progenies, however, usually lead to their dysfunctions or loss *via* the senescence or apoptotic death with advancing age (Figs 1 and 2) [21–28]. This age-related decline in skin regenerative cells may result in a skin homeostatic imbalance and severe cutaneous disorders [23–28]. In particular, a long-term exposure of skin cells to environmental insults such as UV radiations and internal metabolic reactions typically generates highly toxic products and reactive free radicals [1, 3, 21, 23, 25–27, 29, 30]. These molecular events may cause severe damages to mitochondrial and nuclear DNA, membranes, lipids and proteins in skin-resident stem/progenitor cells and their progenies as well as dermal fibroblasts, and ultimately lead to cell dysfunctions or loss during chronological aging. Of therapeutic interest, numerous therapeutic approaches have been developed to restore the skin integrity and functions with advancing age and improve the repair mechanisms after severe injuries. The use of antioxidants, anti-inflammatory and detoxifying agents, calorie restriction and stem cell-based therapies have emerged as the promising strategies to prevent or delay the functional decline or loss of skin-resident adult stem/progenitor cells and their progenies occurring during chronological aging or photoaging [25, 28, 31–43]. These anti-aging strategies, which may improve the stress resistance and survival of skin regenerative cells, represent the potential therapies to prevent the age-related skin detrimental alterations and disorders such as premature aging diseases, chronic non-healing wounds and ulcers and ectodermal dysplasia [25, 28, 31–43].

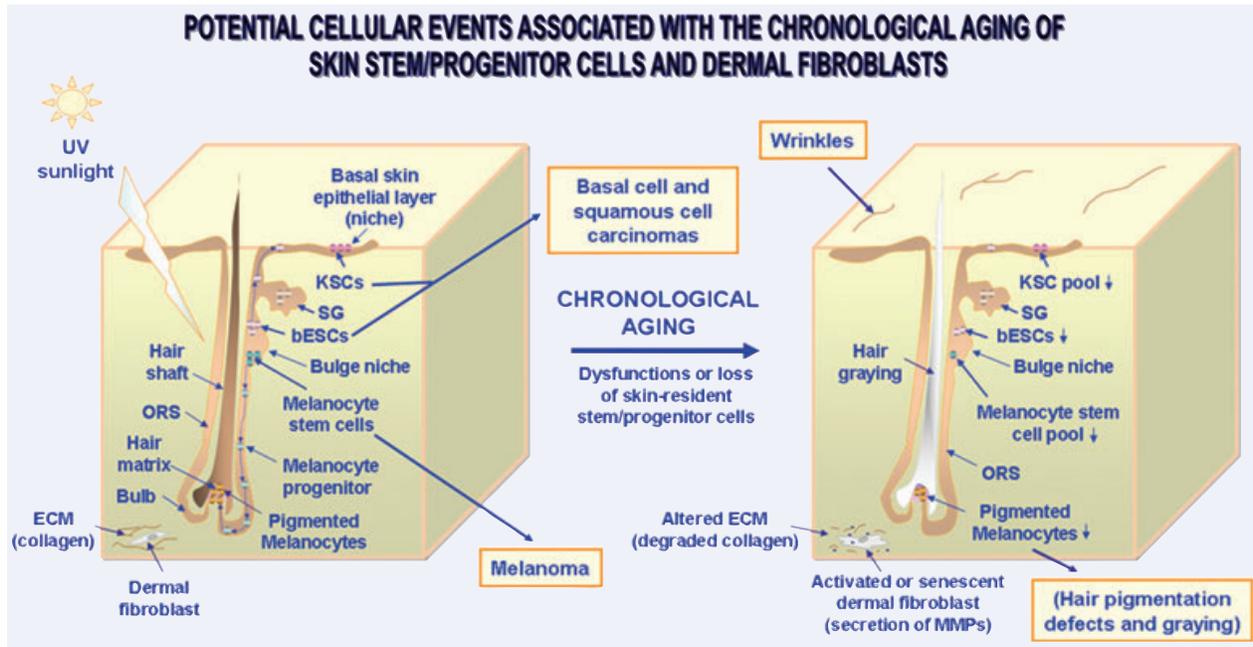
In addition, the photoaging and accumulation of genetic and/or epigenetic alterations in skin stem/progenitor cells and their progenies as well as their microenvironment, including age-related changes in the expression levels of numerous gene products, may lead to development of cutaneous basal cell and squamous cell cancers (BCCs and SCCs) or aggressive and recurrent melanoma (Fig. 1) [1, 4, 6, 13, 22, 28, 44–52]. More specifically, telomere attrition, the gene mutations leading to inactivation of diverse

tumour suppressor gene products [p16<sup>INK4A</sup>, p19<sup>ARF</sup>, retinoblastoma (pRb) and/or p53] combined with a sustained stimulation of diverse developmental signalling cascades initiated by distinct growth factors, and defects in DNA repair mechanisms may contribute to skin cancer initiation and progression (Figs 2 and 3) [1, 6, 13, 28, 44–55]. Among the growth factor cascades that are often deregulated in skin cancer stem/progenitor cells and their progenies, there are hedgehog, epidermal growth factor receptor (EGFR), Wingless (Wnt)/ $\beta$ -catenin, Notch, Nodal/Cripto-1, extracellular matrix (ECM)/integrins and/or Polycomb group protein BIM-1 signalling pathways (Fig. 3). In support with the critical functions of skin cancer stem/progenitor cells in the skin cancer development and metastases, small subpopulations of highly tumorigenic cancer cells with stem cell-like properties have been identified *in situ* and isolated from BCCs, SCCs and primary and secondary melanoma tumours and established skin cancer cell lines [44, 56–66]. In addition, the development of skin cancers is generally accompanied by extensive changes in the local microenvironment of skin cancer stem/progenitor cells and their progenies including in their niches and ECM components as well as the secretion of diverse paracrine mitotic factors by activated stromal cells that contribute to their acquisition of a more malignant behaviour (Fig. 1) [1, 2, 28, 67–69]. Therefore, the molecular targeting of distinct deregulated oncogenic products in skin cancer stem/progenitor cells and their microenvironment offers great promise to eradicate the total cancer cell mass including skin cancer-initiating cells and their progenies, and thereby improve the current cancer therapies.

In this article, we reviewed the specific functions provided by each adult stem/progenitor cell type in skin maintenance and regeneration as well as their potential implications in the development of age-related skin disorders and cancers. The emphasis is on the molecular and cellular mechanisms associated with dysfunctions or loss of skin-resident adult stem/progenitor cells and their progenies during chronological aging as well as new anti-aging and cancer therapies to treat a diversity of severe skin disorders and aggressive cancers.

## Functions of skin-resident adult stem/progenitor cells in skin regeneration

The cellular regeneration of the skin is maintained by different adult stem/progenitor cell subpopulations localized within the specialized microenvironments, niches in interfollicular epidermis (IFE), sebaceous gland and hair follicle bulge region (Fig. 1) [7–20, 70–72]. The epidermal barrier is affected by a continuous loss of terminally differentiated keratinocytes in the outermost epidermal layer, the stratum corneum that are shed from the skin surface during the desquamation process [1, 6]. The small clusters of

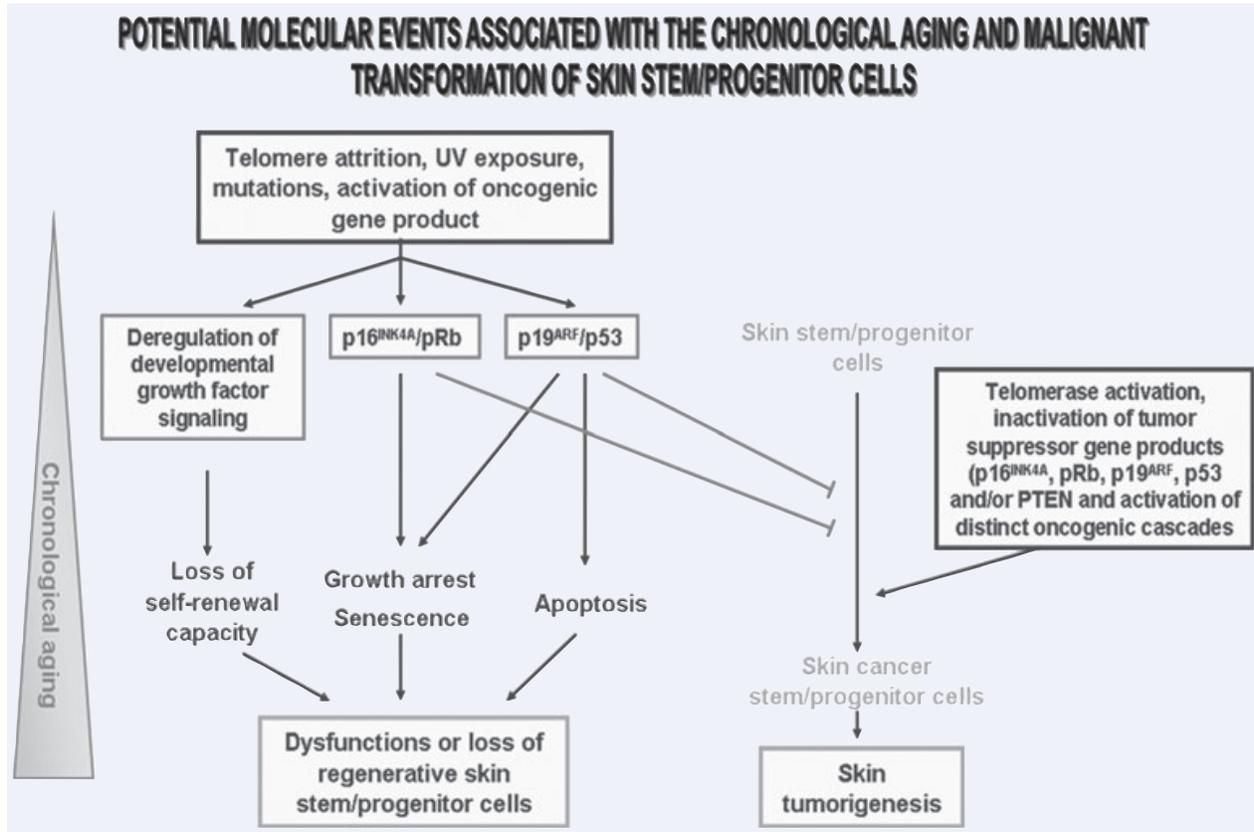


**Fig. 1** Scheme showing the anatomical localization of skin-resident adult stem/progenitor cell niches and the potential cellular changes associated with the skin aging and cancers. The small pool of KSCs resident in basal layer of the epithelial compartment are able to regenerate the skin stratified epithelium by replenishing all of the differentiated epithelial keratinocytes, including terminally differentiated keratinocytes at the skin surface lost during desquamation process. Moreover, bESCs in the follicle hair may also replenish the keratinocytes constituting of appendages including sebaceous gland and inter-follicular epithelium during the hair growth cycle and tissue regeneration after severe skin injury. Additionally, the bulge-resident melanocyte stem cells may generate the melanocyte precursors that can migrate *via* ORS to the germinal matrix at the bulg areas of each hair follicle, and thereby replenish the mature pigmented melanocytes that are responsible for the hair pigmentation by transferring melanin to keratinocytes. This scheme also shows the potential consequences of the chronological aging of skin stem/progenitor cells including the wrinkles and hair growth defects leading a decreased hair pigmentation (greying) and loss. Moreover, the skin cancers including basal cell and squamous cell carcinomas and melanoma which may derive from genetic alterations in KSCs, bESCs or melanocyte stem cells are indicated. The degradation of collagen in dermal layer through the release of MMPs by the activated or senescent dermal fibroblasts which may promote the development of skin disorders and invasive cancer subtype is also illustrated.

undifferentiated and unipotent keratinocyte stem cells (KSCs) with stem cell-like properties and expressing cytokeratins (CK5/14/15), p63,  $\alpha_6\beta_4$ - and  $\alpha_3\beta_1$ -integrins, and ATP-binding cassette (ABC) transporters, have been identified near basement membrane in the basal layer of epithelial compartment (Fig. 1) [2, 7, 8, 10, 11, 13, 70, 73]. KSCs provide critical roles in participating to the continuous replenishment of mature keratinocytes of epidermis in homeostatic conditions and after skin injuries [7, 11, 13, 14]. A complex network of signalling cascades such as EGFR, Notch, insulin-like growth factor (IGF-1)/IGF-R1, immunoglobulin-like domains 1 (Lrig1), Myc, transforming growth factor- $\beta$  (TGF- $\beta$ ) and Polycomb-group protein BMI-1 is involved in the stringent regulation of KSCs behaviour [6, 8–11, 70, 74–79]. The integration of these diverse signalling pathways in KSCs may result in their proliferation and/or differentiation into keratinocytes during skin tissue regeneration. In this regard, an asymmetric division of KSCs generates the transit amplifying (TA)/intermediate cells [80]. TA

cells in turn migrate outside the niche and give rise to the intermediate keratinocytes and more committed terminally differentiated keratinocytes constituting various epidermal layers of the skin [80]. The down-regulation of EGFR in KSCs concomitant with an enhanced activity of p53 and expression of its targets Notch1 and p21<sup>WAF1/CIP1</sup> may notably promote the keratinocyte differentiation [81–83]. Moreover, other signalling effectors, including inhibitor of  $\kappa$ B (I $\kappa$ B) kinase- $\alpha$  (IKK- $\alpha$ ), interferon regulatory factor 6 (IRF6) and cell cycle regulator protein stratifin (SFN, also designated as 14–3–3 $\sigma$  protein) also provide critical functions in the regulatory processes controlling keratinocyte proliferation-differentiation switch from a mitotic state to a terminally differentiated state [73, 84–89]. Additional studies are however necessary to establish the precise roles of IRF6, IKK- $\alpha$  and SFN in KSC behaviour *versus* their differentiated progenies.

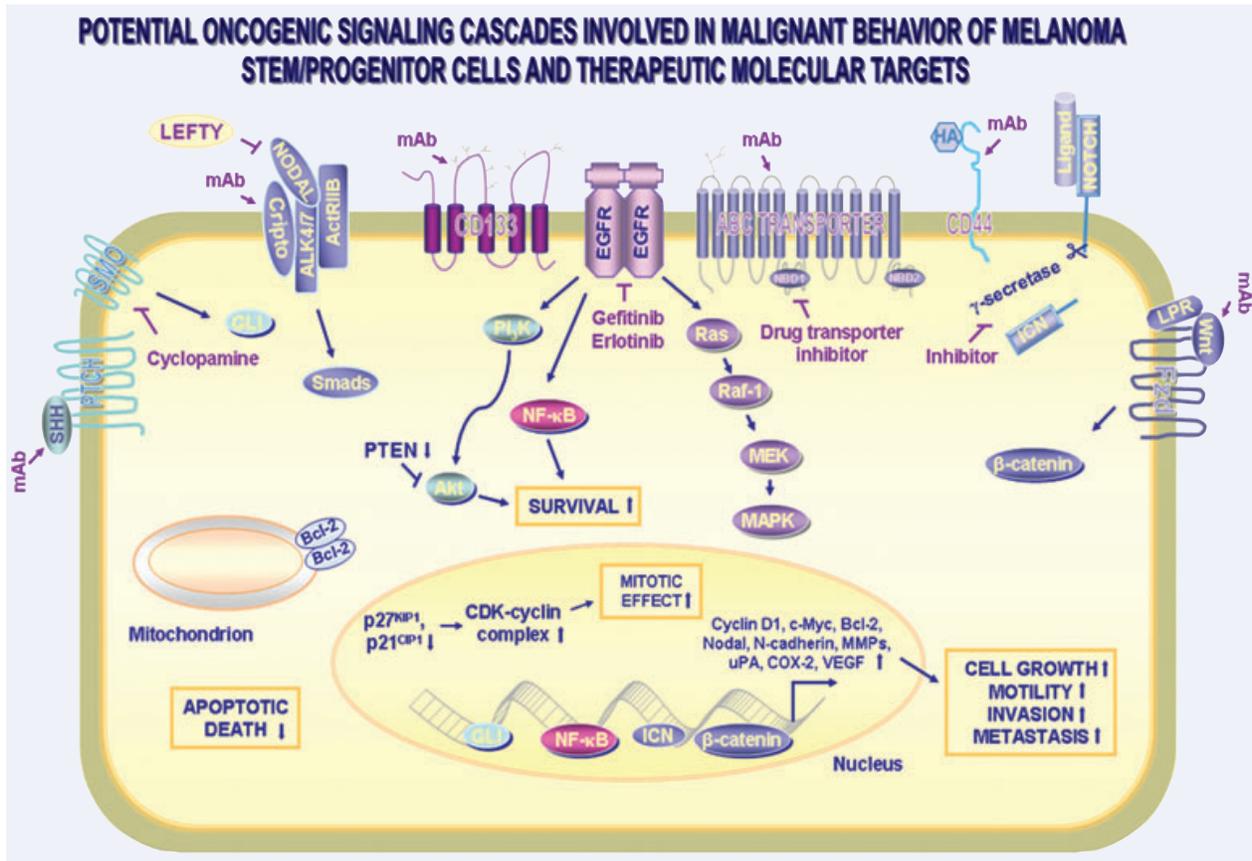
In addition, a small subset of unipotent progenitor cells expressing CK5/14 and B-lymphocyte-induced maturation protein



**Fig. 2** Schematic representation of possible molecular events associated with the dysfunctions or loss of skin-resident stem/progenitor cells. This scheme suggests that telomere shortening, mutations, DNA repair defects and/or a long-term ultraviolet (UV) exposure of skin-resident stem/progenitor cells during chronological aging may be accompanied by a decline of their functions or loss. Particularly, the alterations in the growth factor signalling pathways involved in the stringent control of the self-renewal capacity of these immature cells may lead to their dysfunction. Moreover, the enhanced expression levels and activation of the p16<sup>INK4A</sup>/retinoblastoma (Rb) and p19<sup>ARF</sup>/p53 cascades may result in an inhibition of CDK/cyclin complexes, inactivation of the E2F transcription factor and growth arrest or senescence. In addition, p53-induced pro-apoptotic factors including Bax, may also trigger the apoptotic death of these skin regenerative cells with advancing age. In addition, this scheme also specifies that the tumour-suppressing effect mediated through the p16<sup>INK4A</sup>- and/or p53-initiated pathways may counteract the malignant transformation of skin cancer-initiating cells. Alternatively, the telomerase activation combined with the occurrence of distinct genetic and/or epigenetic alterations leading to inactivation of tumour suppressor genes and sustained activation of diverse developmental pathways in skin stem/progenitor cells may culminate in their malignant transformation into skin cancer stem/progenitor cells and skin tumour formation.

1 (BLIMP1) has also been identified near basement membrane surrounding the cutaneous sebaceous gland in the hair follicle (Fig. 1) [17]. These skin progenitor cells contribute to regenerate the sebaceous gland including the terminally differentiated sebocytes that provide important functions in the endocrine and hormonal regulation of skin integrity and sebum secretion [5, 17]. Moreover, the multipotent epithelial stem cells (bESCs) expressing different markers such as CK5/14/15/19, CD200, p63,  $\alpha_6\beta_4$ - and  $\alpha_3\beta_1$ -integrins have been identified in the bulge niche of the hair follicle (Fig. 1) [8, 9, 12, 15, 16, 22, 71, 90–93]. It has also been reported that murine bESC may be distinguished from KSCs on

the basis of their CD34 expression while the CD34 marker is not expressed by human bESCs in bulge niche [12, 71, 94, 95]. A human CD34<sup>+</sup> subpopulation resident in upper region of the outer root sheath (ORS) of the hair follicle, however, has been observed to more effectively regenerate new hair follicle in immunodeficient nude mouse than CD34<sup>-</sup> cells suggesting that a large niche could be occupied by human ESCs [96]. bESCs or early progenies serve as a source of immature cells to regenerate the epidermal cells in skin appendages or damaged epithelial tissue in response to intense injuries (Fig. 1) [2, 7–9, 12–16, 22, 71, 92–94]. Indeed, the hair follicles typically undergo repeated growth cycles consisting



**Fig. 3** Scheme showing the potential oncogenic cascades involved in the malignant and aggressive phenotypes of melanoma stem/progenitor cells. The intracellular signalling cascades induced through the activation of distinct growth factor pathways which may provide a critical role for the sustained growth, survival, migration, invasion, metastases and/or drug resistance of melanoma stem/progenitor cells, are shown. These tumorigenic cascades include sonic hedgehog SHH/PTCH (patched receptor)/GLI, Cripto-Nodal-ALK4/7/ActRIIB activin-like kinases receptor complex, CD133 pentaspan transmembrane protein, Notch/Ligand (Delta and Jagged), hyaluronan (HA)/CD44 and Wnt/Frizzled (Fzd)/ $\beta$ -catenin. The up-regulated expression levels of certain target gene products including up-regulated cyclin D1, c-Myc, Bcl-2, Nodal, N-cadherin, matrix metalloproteinase (MMPs), urokinase plasminogen (uPA), cyclooxygenase (COX-2) and vascular epidermal growth factor (VEGF) that can contribute to the malignant transformation of melanoma stem/progenitor cells are also indicated. In addition, the potential molecular targeting strategies by using a selective inhibitor of SMO hedgehog signalling element (cyclopamine), Nodal (Lefty), EGFR (gefitinib or erlotinib),  $\gamma$ -secretase and monoclonal antibody (mAb) directed against Cripto-1, CD133 and ABC multidrug transporter.

to the periods of active growth (anagen), regression (catagen) and rest (telogen). During the hair cycle, the activation of quiescent bESC subpopulation leads to their division and generation of TA cells that in turn can leave the bulge niche and migrate along the ORS forming the outermost layer toward germinal matrix and dermal papilla where they can give rise to mature keratinocytes (Fig. 1). In this matter, it has been reported that the induction of telomerase or its catalytic subunit, telomerase reverse transcriptase (TERT) in mouse skin epithelium caused a rapid transition from the resting phase of hair follicle cycle (telogen) into the active phase (anagen) by activating hair follicle stem cell proliferation [97–100]. In fact, TERT may directly promote the entrance of

quiescent hair follicle stem/progenitor cells into the cell cycle by modulating the Wnt and Myc pathways [98–101]. The interplay of a complex network of several developmental signalling cascades initiated by different growth factors as well as the changes in key niche components generally participate to the stringent regulation of bESC behaviour during hair follicle growth and/or IFE regeneration after severe injuries [13, 15, 22, 71, 93, 102–106]. These pathways include sonic hedgehog (SHH/PTCH/GLI), EGFR, Wnt/ $\beta$ -catenin, Notch, nuclear factor-kappa B (NF- $\kappa$ B), bone morphogenic protein (BMP), BMI-1 and SOX9 transcription factor. Particularly, the activation of Wnt/ $\beta$ -catenin pathway seems to be necessary for the fate specification of bESCs into hair follicular

keratinocytes rather IFE keratinocytes during hair growth cycle while Notch signalling may contribute to the maintenance of the hair follicle fate of bESCs [103, 107–110].

The lower permanent portion of the hair follicle bulge region in human and mice also contains a small subpopulation of immature melanocyte stem cells with stem cell-like properties that can give rise to melanocyte precursors and mature melanocytes in bulb areas at the base of hair follicle (Fig. 1) [18, 21, 111–115]. The gene expression analyses of melanocyte stem cells isolated from murine hair follicles revealed that they express dopachrome tautomerase (Dct) and paired-box transcription factor PAX3 like melanocytes [111, 115]. The undifferentiated melanocyte stem cells, however, may be distinguished from melanocyte precursors and mature melanocytes by a down-regulated expression of melanocyte-specific gene products such as microphthalmia-associated transcription factor (MITF), SRY (sex determining region Y)-box 10, SOX10 transcription factor, lymphoid enhancer-binding factor 1 (LEF1), KIT and tyrosinase-related protein 1 (Typr1) [111, 115]. More specifically, during the anagen phase of hair follicle cycle, activated melanocyte stem cells in the hair follicle bulge region generate the melanocyte precursors that migrate along the ORS to regenerate the epidermal and hair matrix melanocytes (Fig. 1) [18, 21, 112]. The differentiated and pigmented melanocytes localized at the base of each hair follicle in the bulb areas provide critical functions in hair pigmentation by producing the specialized melanin-containing vesicles, melanosomes that are transferred to neighbouring keratinocytes. Numerous signalling pathways are involved in the stringent regulation of the maintenance of bulge-resident melanocyte stem cells in quiescent *versus* activated states as well as in their survival, proliferation, migration and differentiation into melanocyte lineage [18, 21, 113–123]. These signalling elements include Notch1 and 2, stem cell factor (SCF)/KIT, stromal cell-derived factor-1 (SDF-1)/CXCR4, PAX3, MITF and anti-apoptotic factor, Bcl-2 [18, 21, 113–123].

In addition, different cell types, which may derive from pluripotent neural crest stem cells during embryonic development, may also be generated from bulge-resident stem cells or their progenitors in post-natal life in adult mammals [19, 20, 113, 124–132]. More particularly, the bulge area in human hair follicle contains a small subpopulation of pluripotent epidermal neural crest stem cells (eNCSCs) expressing stem cell-like markers such as nestin, SOX9, Nanog and Oct-3/4 that can give rise to multiple skin cell lineages including melanocytes, neurons and smooth muscle cells [20]. Another subpopulation of multipotent adult skin-derived precursors (SKPs), which reside within the dermal papillae of hair follicles, also appear to exhibit properties similar to those of eNCSCs [129, 130, 133]. SKPs can be expanded in the presence of EGF, basic fibroblast growth factor (bFGF) and TGF- $\beta$  and give rise to neurons, glia, smooth muscle cells, and adipocytes in culture *in vitro*. Additionally, it has also been reported that the multipotent human skin-derived CD133<sup>+</sup> cells expressing the CD34 and Thy-1 antigens were able to proliferate in the presence of EGF and bFGF, form spheres, and differentiate into neurons, astrocytes, and rarely into oligodendrocytes *in vitro* as well as astrocytes and endothelium *in vivo* [134].

Future investigations are however necessary to establish the hierarchical organization of these skin-resident stem/progenitor cells, and more particularly, to determine whether bulge-resident melanocyte stem cells or SKPs may arise from more primitive eNCSCs as well as their specific functions in skin maintenance and cell regeneration after severe injuries.

On the other hand, the stromal cells including fibroblasts and dermal sheath cells (DSCs) as well as multipotent dermal-resident mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs) have been detected in human dermis and vascular wall of skin tissue, respectively [78, 135–137]. Hence, these skin cells can participate to skin regeneration and cutaneous wound healing through the release of diverse paracrine factors. More specifically, the differentiation of EPCs into mature endothelial cells may contribute to the formation of new vessels after severe skin injuries. Similarly, bone marrow (BM)-derived MSCs and EPCs can also migrate to severe injured cutaneous tissues such as non-healing chronic wounds, and thereby take part to skin repair and regeneration processes [135, 138, 139].

Collectively, these observations suggest that the interplay of different types of skin-resident adult stem/progenitor cells contribute to the maintenance of skin integrity and functions in homeostatic conditions and during tissue repair after severe injuries. Unfortunately, the dysfunctions, loss or malignant transformation of these rare regenerative skin-resident stem/progenitor cells during chronological aging or in pathological conditions may result in the development of severe skin disorders and cancers.

## Altered functions of skin-resident adult stem/progenitor cells during chronological aging

The structural integrity and functions of skin are generally altered with passage of time [1, 3, 21, 25]. The chronological intrinsic aging of skin cells may result in a progressive decrease of turnover epidermal time and loss of tone and elasticity of skin [1, 3, 21, 25, 140]. Moreover, the skin aging is also accompanied by an increased susceptibility to injury and infection, epidermal dehydration, reduced wound healing, wrinkles (rhytides), pigmentary alterations, hair greying and loss, blood vessels weakness, and increased risk of skin epithelial cancers and melanoma (Fig. 1) [1, 3, 21, 25–27, 140]. Among the principal environmental factors that may promote the skin-aging process and ultimately carcinogenesis, a repeated exposure to solar UV radiations is critical [25, 27, 48, 49, 54, 141]. Although the active repair mechanisms and melanin synthesis may protect the skin cells against UV radiation-induced cellular damages, a chronic or cumulative exposure to sunlight may induce several gene mutations and culminate to development of skin disorders such as epidermal

atrophy, psoriasis, keratosis, depigmentation and dysplasia as well as skin cancer initiation.

## Potential implications of skin-resident stem/progenitor cells in skin aging-related disorders

Accumulating lines of experimental evidence have revealed that the chronological or premature aging of skin may be associated with dysfunctions or loss of certain skin-resident stem/progenitor cells (Figs 1 and 2) [24, 26–28, 97]. This decline in the functional properties or number of KSCs and bESCs with the time may impair the skin regeneration process and wound healing, and contribute to development of diverse skin disorders [10, 24, 26, 27, 97]. For instance, the results from a study carried out using the sun-exposed and sun-protected skin samples obtained from old individuals revealed that the KSC number was lower in photoaged skin than in chronologically aged skin specimens [27]. Moreover, it has been reported that the telomere shortening may occur in hair follicle stem cells with aging, and thereby lead to DNA instability and a decline in their functional properties including their capacity to replenish mature and functional keratinocytes and melanocytes of follicle during the hair growth cycle [97].

The chronological aging and photoaging of skin is also accompanied by an extensive ECM remodelling in dermal layers such as the occurrence of EMT program or senescence of skin fibroblasts, dramatic up-regulation of matrix metalloproteinases (MMPs) and a decrease of collagen production (Fig. 1) [140, 142, 143]. Particularly, the fibroblasts in aged skin can produce high levels of matrix MMPs and low levels of collagen [142]. An exposure of human skin fibroblasts to UV irradiation may also result in oxidative stress and enhanced expression of p16<sup>INK4A</sup> and p53/p21<sup>WAF1/CIP1</sup>-induced fibroblast premature senescence and/or the activation of the fibroblast to myofibroblast transition [29, 69, 143, 144]. These molecular events may lead to a loss of skin tone and elasticity, fibrosis and increased risk to develop aggressive and invasive skin cancers such as melanoma and morphoeic BCC subtype, which is characterized by an infiltrative growth pattern and densely fibrous stroma as compared to the most low-risk and indolent BCCs [29, 68, 69, 144].

In addition, diverse premature aging disorders, which are typically caused by inherited or sporadic genetic mutations in key gene products resulting in detrimental nuclear lamina alterations, an accelerated telomere attrition and/or DNA repair defects, are also associated with an accelerated apparition of skin aging phenotypes and premature death of patients [145–152]. The premature aging disorders include Hutchinson-Gilford progeria syndrome, Werner syndrome, dyskeratosis congenital, ataxia-telangiectasia, Fanconi anaemia, aplastic anaemia, xeroderma pigmentosum (XP), Cockayne syndrome and trichothiodystrophy [145–153]. In general, the genetic abnormalities associated with these diseases may lead to genomic instability and dysfunctions in adult stem/progenitor cells and their progenies in diverse tissues including skin. Hence, these accel-

erated aging disorders may be accompanied by a premature loss of hair and subcutaneous fat, hair greying, severe skin atrophy and/or an enhanced propensity to develop diverse diseases including skin disorders and cancers [28, 148–161]. In this regard, several animal models have been developed to establish the cellular and molecular events that may promote the aging process and skin disorder development.

## Animal models of skin-resident stem/progenitor cell aging and disorders

Several studies carried out on mice models have indicated the potential implication of the senescence or apoptotic death of skin-resident adult stem/progenitor cells and their progenies in acquisition of certain premature aging phenotypes and the pathophysiological development of age-related skin disorders [21, 97, 116, 119, 152, 162]. For instance, it has been observed that the telomerase-deficient *TERC*<sup>-/-</sup> mice with critically short telomeres, which possess dysfunctional bESCs showing a reduced ability to be mobilized out of their niche and an impaired capacity to regenerate the hair follicle and skin epidermis, were characterized by a premature skin-aging and death [98]. In fact, bESCs isolated from these telomerase-deficient mice exhibited a low proliferative capacity *ex vivo* [98]. Interestingly, the *TERT* re-introduction into late-generation telomerase-deficient *TERC*<sup>-/-</sup> mice, however, could restore the telomerase activity and telomere length and prevent bESC defects and premature skin pathologies associated with aging and death of mice without increased the tumorigenesis [98, 163]. In the same pathway, it has also been reported that the number and proliferative capacity of bESCs were decreased in *Zmpste24*-null progeroid mice, which display progeroid symptoms characterized by an aberrant nuclear envelope architecture due to nuclear lamina defects, as compared to *Zmpste24*<sup>-/-</sup> *Lmna*<sup>+/-</sup> mutant mice [164]. It has also been noticed that *Wnt* and *MITF* were altered in dysfunctional bESCs and these immature cells and their supporting cells in the hair bulb region showed an enhanced rate of apoptotic death [164]. The *p53*<sup>+/*m*</sup> or  $\Delta$ *Np63 $\alpha$*  mice, which display the pathological signs associated with premature skin aging-related phenotypes, were also characterized by a decreased expression level of nicotinamide adenine dinucleotide (NAD)-dependent deacetylase protein, sirtuin 1 (*SIRT1*, silent mating type information regulation 2, homologue 1), and reduced longevity relative to wild-type animal [165]. Importantly, an inhibition or conditional ablation of *Notch/Hes1*, *Bcl-2*-dependent *SCF/KIT* and master regulator *MITF* signalling elements also caused a gradual and dramatic elimination of bulge-resident melanocyte stem/progenitor cells by apoptosis in animal model *in vivo* [21, 112, 118–120, 126, 166]. This progressive loss of melanocyte stem/progenitor cells and their mature progenies, the melanin-producing melanocytes in the bulb areas, was accompanied by a severe defect in hair pigmentation and an accelerated and intensive hair greying, the prototypical signs of skin aging (Fig. 1)

[21, 118–120, 166]. Of particular interest, the hair greying phenotype and premature mice death by polycystic kidney disease observed for *Bcl-2*<sup>-/-</sup> mice could be prevented by a simultaneous down-regulation of pro-apoptotic BH3-only Bcl-2 family member, BIM suggesting that this factor may constitute a potential anti-aging therapeutic target [166].

In addition, certain lines of experimental evidence have also indicated that the activation of p16<sup>INK4A</sup>/pRb and/or p53 tumour suppressor-induced signalling pathways, which may lead to cellular senescence or apoptotic death, may contribute to counteract skin cancer development (Fig. 2) [28, 167–171]. As a matter of fact, the presence of functional p53 signalling pathway in *TERC*<sup>-/-</sup> mice, which are deficient in telomerase, has been associated with an induction of senescence and apoptotic cell death and enhanced resistant to spontaneous skin tumorigenesis [170, 171]. However, *TERC*<sup>-/-</sup> p53<sup>R172P</sup> mice, which are able to trigger the senescence via the p53/ p21<sup>CIP1/WAP1</sup> cascade but not apoptosis were resistant to spontaneous skin tumorigenesis while skin carcinomas were induced by 7, 12-dimethylbenz[a]anthracene (DMBA) plus 12-O-tetradecanoylphorbol-13-acetate (TPA) in these mice [171]. In fact, five out of seven chemically treated *TERC*<sup>-/-</sup> p53<sup>R172P</sup> mice developed highly proliferative pre-malignant lesions, squamous papillous/keratoacanthomas and four out of five mice displayed invasive SCCs despite a high staining of p53/p21<sup>CIP1/WAP1</sup> were detected in these malignant lesions [171]. In the same way, it has been observed that the *K5TRF2* mice overexpressing telomere-binding factor 2 (TRF2) under control of the regulatory region of keratin 5 (K5) gene in the stem cell compartment of different epithelia including the skin, exhibited severe premature skin aging defects with increased age as compared to age-matched wild-type mice [152, 172]. These skin defects of *K5TRF2* mice include hair loss, skin hyperpigmentation and dryness and an increased predisposition to develop skin cancer after exposure to light [152, 172]. These skin defects, which resemble to the skin phenotypes observed in some human premature aging disorders such as XP syndrome, have been associated with a XPF nuclease-dependent telomere loss and increased chromosomal instability induced through an enhanced expression of TRF2 and p53 in bESCs leading to their dysfunction [152]. Importantly, p53 deletion in *K5TRF2* mice model could rescue bESC defects in the absence of telomere elongation while these *K5TRF2/p53*<sup>-/-</sup> mice showed an accelerated onset of pre-neoplastic skin lesions and UV-induced skin carcinogenesis including BCCs and SCCs relative to *K5TRF2*, *p53*<sup>-/-</sup> and/or wild-type mice used as controls [152].

Altogether, these observations suggest that the induction of cell cycle arrest and senescence mediated through the stimulation of tumour suppressor gene products such as p53 may be effective to decrease the incidence of only certain skin cancer subtypes. Moreover, the down-regulation of tumour suppressor gene products combined with an accumulation of numerous genetic and/or epigenetic alterations in skin-resident stem/progenitor cells during chronological aging or photoaging may also allow them to bypass the senescence and apoptotic cell death processes and culminate to skin cancer development.

## Altered functions of skin-resident adult stem/progenitor cells in skin hyperproliferative disorders and cancers

The down-regulation of tumour suppressor gene products such as p16<sup>INK4A</sup>/pRb, p19<sup>ARF</sup>/p53/p21<sup>WAP1/CIP1</sup> and/or phosphatase and tensin homologue deleted on chromosome 10 (PTEN), deregulation of growth factor signalling cascades involved in the stringent regulation of proliferation, survival and/or differentiation of KSCs and bulge stem cells and photoaging represent important factors that contribute to development of diverse skin pathologies (Figs 1 and 2) [6, 51, 55, 83, 173–176]. Among them, there are the epidermal hyperproliferative disorders such as impaired wound healing and psoriasis, basaloid follicular hamartoma and skin cancers including BCCs, SCCs and melanoma. Particularly, a long-term skin exposure to UV radiations may cause inflammatory and immunosuppressive effects and DNA damages on sun-exposed areas of skin, and thereby result in major DNA mutations and photoproduct formation in skin cells promoting skin cancer initiation [25, 48–50, 52, 162, 177]. For instance, a depletion of cellular antioxidant enzymes such as catalase and superoxide dismutase (SOD) induced through an UV light exposure may result in an enhanced reactive oxygen species (ROS) production and oxidative stress [25, 30]. These molecular events may cause severe damages to DNA, proteins, lipids and carbohydrates in skin cells, and thereby promote skin cancer development. In this matter, we are reporting frequent deregulation occurring in skin-resident adult stem/progenitor cells and their progenies which may be associated with the development of BCCs, SCCs or melanoma.

## Potential implications of cancer stem/progenitor cells in basal cell and squamous cell cancers

Recent studies revealed that an extensive UV light exposure-induced DNA mutations and genetic and/or epigenetic alterations leading to a sustained activation of TERT, hedgehog, EGFR and/or Wnt/ $\beta$ -catenin signalling pathways in skin-resident stem/progenitor cells and their progenies contribute to the development of certain cutaneous BCC and SCC subtypes [1, 49, 52, 83, 173, 178]. In this regard, accumulating lines of experimental evidence have indicated that certain subtypes of BCCs and SCCs could derive from the malignant transformation either of skin bESCs or their early progenies localized within or in close proximity to the bulge niche in hair follicles and/or skin cells in the basal layer of IFE [44, 47, 56, 57, 175, 179, 180]. More particularly, a sustained activation of sonic hedgehog pathway SHH/patched (PTCH)/GLI transcription factors, due to an overexpression of SHH ligand, activating mutations in smoothed (SMO) co-receptor or inactivating mutations in PTCH receptor as well as Wnt/ $\beta$ -catenin cascade in skin cells has been associated with the etiopathogenesis of certain inherited or sporadic BCC

subtypes in human [49, 102, 180–184]. Consistent with critical implication of hedgehog cascade in BCCs, it has been shown that an aberrant expression or activation of hedgehog signalling effectors, including the overexpression of SHH ligand, an activated human M2SMO mutant or GLI-1 or 2, in skin stem/progenitor cells found in hair follicles and/or basal layer of epidermis in mice model *in vivo* resulted in the cell hyperproliferation and human BCC-like tumour development [56, 175, 185–192]. Moreover, it has been observed that the SOX9 transcription factor, which represents a potential downstream target of hedgehog pathway in hair follicle bESCs, was strongly expressed in cancer cells detected in BCCs [179]. The ablation of Notch1 receptor in mouse epidermis by using tissue-specific inducible gene-targeting approach also resulted in a sustained expression of GLI-2 concomitant with the development of epidermal hyperplasia followed by BCC-like tumours [193]. These data suggest then that Notch1 may act as a tumour-suppressor gene product in skin epidermis. In this matter, loss-of-function mutations in PTCH-1 receptor or activating mutations in SMO resulting in a constitutive activation of hedgehog pathway, has also been associated with the development of rare diseases such as nevoid basal cell carcinoma syndrome (NBCCS) and accelerated aging disorder, XP, which are typically characterized by skin defects and a high BCC incidence [159–161, 183, 186, 194].

On the other hand, an increased expression of CK19, which is considered as a putative marker of bESCs, has also been observed in certain SCC tissue specimens [44]. Moreover, a small subpopulation of murine CD34<sup>+</sup> cancer stem/progenitor cell population characterized by the phenotypic and functional properties like normal bESCs has been detected in early stage of SCCs [57]. The isolated fraction of CD34<sup>+</sup> cancer stem/progenitor cells were able to give rise to the tumours after transplantation in mice *in vivo* showing a hierarchical organization resembling primary malignant neoplasm [57]. The ablation of the  $\beta$ -catenin gene in these CD34<sup>+</sup> cancer stem/progenitor cells resulted in their loss and a complete tumour regression suggesting a critical role of the activation of Wnt/ $\beta$ -catenin cascade in triggering of certain SCC subtypes [57]. The transcriptional suppression of p53 induced-Notch gene expression through the activation of EGFR/c-Jun signalling in keratinocytes also led to a down-regulation of Notch1-initiated differentiation pathway and expansion of stem cell populations that promoted the development of SCCs [82, 83]. In spite these advances, additional investigations are necessary to more precisely determine the specific implication of KSCs residing near the basement membrane in basal epithelial layer *versus* bESCs in the development of different BCC or SCC subtypes (Fig. 1).

### Potential implications of cancer stem/progenitor cells in melanoma

A growing body of experimental evidence has also revealed that the cutaneous melanoma, which generally shows a high propen-

sity to metastasize and resistance to current therapeutic treatments, could arise from cancer cells endowed with stem cell-like properties [58–63, 112, 195–198]. In support with this, small subpopulations of cancer stem/progenitor cells with stem cell-like properties have been identified and isolated from primary and secondary tumour specimens of patients with melanoma as well as human primary WM115 and metastatic WM239A and murine B16F10 melanoma cell lines [58–63, 65, 195, 196, 198]. These melanoma stem/progenitor cells expressed different biomarkers [CD133, CD44, CD20, telomerase reverse transcriptase (TERT), nestin, Nanog and/or ABC transporters such as MDR-1, ABCG2 and ABCB5]. More specifically, it has been reported that a small subset of cancer stem/progenitor cells expressing MDR-1/ABCB2/ABCB5/Nanog/TERT markers, which has been isolated from primary melanoma specimens, showed higher clonogenicity and self-renewal capacities *in vitro* than MDR-1 negative cancer cell fraction [62]. The *ex vivo* culture of a CD20<sup>+</sup> cancer cell fraction enriched from melanoma cell lines and metastases in the growth medium, which is used for human embryonic stem cells, also resulted in their propagation under form of non-adherent spheres [59]. Each individual multipotent CD20<sup>+</sup> cancer cell from melanoma spheres was able to differentiate like eNCSCs into multiple cell types showing the phenotypic features like the melanocytes, adipocytes, osteocytes and chondrocytes under well definite culture conditions [59]. Moreover, the CD133<sup>+</sup> melanoma cells isolated from metastatic melanoma biopsies were also able to form melanocytic tumours in mice *in vivo* while the CD133<sup>-</sup> cancer cell fraction does not induce the melanoma formation [61]. In the same way, a small subpopulation of Cripto-1<sup>+</sup> cells expressing elevated levels of Oct3/4, Nanog and MDR-1 stem cell-like markers, which has been isolated from the metastatic melanoma cell line C8161, also displayed a slow growing and sphere forming capacity *in vitro* [199]. Another small side population (SP) expressing nestin and premelanosomal marker, gp100 has also been isolated by efflux Hoechst 33342 dye technique from metastatic melanoma DM1N, DM2N and DM3N cell lines which were originally established from lymph node melanoma metastases. It has been observed that these SP cells, which possessed a small size and were less melanized than other cell fraction, were able to give rise to differentiated melanoma cells. These SP cells also showed a high resistant to cytosine  $\beta$ -D-arabino-furanoside hydrochloride (Ara-C) treatment [64]. Hence, it appears that the primary and metastatic melanomas may arise from tumorigenic cancer cells with the stem cell-like properties. Particularly, the transition from skin-confined melanoma into invasive and metastatic melanocytic tumours usually implicates an accumulation of distinct oncogenic events in tumorigenic melanoma cells.

### Molecular events associated with melanoma progression

The progression of primary melanoma into aggressive, invasive and metastatic melanocytic tumours is usually associated with a deregulation in numerous signalling elements. Among them,

there are the alterations in tumour suppressing pathways including p16<sup>INK4A</sup>/cyclin-dependent kinase 4 (CDK4)/pRb and p19<sup>ARF</sup>/p53 as well as the activation of multiple growth factor cascades such as hedgehog, Wnt, Notch, Nodal and diverse receptor tyrosine kinases (RTKs) such as EGF/EGFR, SCF/KIT and hepatocyte growth factor/scatter factor (HGF)/c-MET and activating mutations in KIT (Fig. 3) [46, 53, 66, 174, 200–204]. Particularly, inherited or somatic events including an inactivation of tumour suppressor genes such as p16<sup>INK4A</sup>, p19<sup>ARF</sup>, p53 and PTEN and/or activating mutations in KIT, H-Ras, N-Ras or B-Raf (v-Raf murine sarcoma viral oncogene homologue B1) combined with a sustained activation of diverse signalling elements including Ras/ERK, PI<sub>3</sub>K/Akt and NF- $\kappa$ B generally contribute to melanoma development (Figs 2 and 3) [55, 177, 201, 205–210]. For instance, the data from an investigation consisting of a 10-year follow-up period of 31 patients with melanoma have revealed that a decreased expression of pRb, p53, p21<sup>WAF1/CIP1</sup>, p27<sup>KIP1</sup> and pro-apoptotic factors, Bax and Bak may be associated with a higher risk of melanoma metastases [55]. In support with critical functions of p16<sup>INK4A</sup> and p19<sup>ARF</sup> protein loss and Ras activation in the development of aggressive metastatic melanomas, it has been observed that the majority of Tyr::N-Ras<sup>Q61K</sup> INK4A<sup>-/-</sup> transgenic mice developed primary melanocytic and multifocal tumours that metastasized to lymph nodes, lung and liver [66]. The cancer cells expressing the stem cell-like marker, nestin isolated from primary melanoma specimen could also be subcutaneously transplanted in nude mice, and when intravenously injected into non-obese diabetic-severe combined immunodeficiency (NOD/SCID) mice, they metastasized at lung [66]. Interestingly, recent data have also revealed that the stem cell-like markers including CD133, CD44 and ABCB5 multidrug transporter, which are expressed by melanoma stem/progenitor cells, can contribute to their malignant behaviour, and thereby they could constitute the potential molecular therapeutic targets (Fig. 3) [63, 65, 195]. In this matter, it is worth mentioning that the phenotypic and functional features of melanoma stem/progenitor cells as well as the number of melanoma-initiating cells that are able to drive tumour development may vary during the disease progression and before and after treatment initiation. As a matter of fact, a high proportion of human melanoma cells isolated from patients with primary and metastatic melanomas and expressing different phenotypic markers were able to form tumours in highly immunocompromised mice models *in vivo* [211]. Therefore, future investigations are required to more precisely define the gene expression profiles and functions of specific stem cell-like markers expressed by melanoma stem/progenitor cells *versus* their differentiated progenies in the primary and secondary melanocytic tumours during cancer etiology and progression. Particularly, it will be important to establish if melanoma-initiating cells may derive from the malignant transformation of bulge-resident stem/progenitor cells like eNCSCs, melanocyte stem cells and/or their early progenitors endowed with the stem cell-like properties. These studies should help to develop effec-

tive multitargeted therapies for eradicating the total mass of melanoma-initiating cells and their progenies at early and late stages of disease.

Collectively, together these observations suggest a major implication of dysfunctions or loss of skin-resident adult stem/progenitor cells in the development of numerous age-related skin disorders and carcinogenesis. These studies support then the therapeutic interest to target these immature and dysfunctional cells to prevent or treat diverse skin disorders and aggressive skin cancers that may be promoted with the passage of time. In regard with this, we described new promising anti-aging and cancer therapeutic strategies consisting of stem cell-based therapies and molecular targeting of deregulated signalling elements in skin cancer-initiating cells to treat diverse age-associated skin diseases and cancers in human.

## Novel stem cell-based therapies

### Novel anti-aging therapies

#### Modulation of aging process by calorie restriction and pharmacological agents

Numerous studies have revealed that a nutritious diet, also designated as calorie restriction, use of pharmacological agents including anti-inflammatory and antioxidant compounds and activators of protein deacetylases such as sirtuins (silent information regulators) may represent the potent therapeutic strategies for improving cell survival and treating diverse metabolic skin disorders [25, 31–34, 212–214]. In fact, calorie restriction or anti-aging skin cares with these agent types may influence the expression and/or activity of signalling elements known to be involved in inflammation, oxidative stress, senescence or apoptotic death of skin-resident adult stem/progenitor cells and their progenies during chronological aging [25, 31, 32]. More specifically, the use of activators of sirtuins, which act as NAD<sup>+</sup>-dependent protein deacetylases, may modulate the activity of key gene products including p53 and forkhead box O (FOXOs) family of transcription factors that are involved in the regulation of cellular metabolism, stress resistance, senescence and apoptosis. Thereby, the activators of sirtuins can promote the cell survival and lifespan extension [31, 32, 215]. For instance, the results from a study consisting in a topical application on the face and neck of a formulation containing 1% yeast Kluyveromyces biopeptides acting as a sirtuin 1 activator carried out with 33 women aged from 37 to 64 years, indicated that this anti-aging skin care significantly improved the hydration, firmness and pigment colour intensity of skin after 4 weeks of treatment [31]. Moreover, the application of this formulation enriched in yeast Kluyveromyces biopeptides, which increased the expression levels of nuclear longevity protein sirtuin 1, also decreased the rate of senescence and apoptotic death induced by UVB exposure on *ex vivo* epidermal cell culture

established from healthy human skin [31]. In the same way, the use of natural polyphenolic compound, resveratrol (trans-3, 4', 5-trihydroxystilbene) found in red wines and diverse plant species such as grapes, peanuts, berries and pines, as a skin care ingredient, has also been shown to induce the anti-inflammatory and antioxidant effects and enhance the sirtuin enzymatic activity [33–37, 216, 217]. More specifically, a topical application of resveratrol may reduce cellular ROS levels and prevent the development of cutaneous disorders such as skin hyperplasia and cancers that may be caused by an UVB light exposure and metabolic stress during chronological aging [34, 36, 37, 216, 217]. Although the therapeutic interest to use resveratrol in skin care preparations, it has been reported that this agent, in counterpart, can also potentiate certain types of UVA-induced DNA strand breaks and apoptotic death of immortalized HaCat human keratinocyte cell line *in vitro* [218]. On the other hand, the activation of residual hair bulge-resident melanocyte stem/progenitor cells and/or their early progenies also may constitute a potential strategy for recovery melanin-producing melanocytes in hair bulb areas and treatment of hypopigmentation disorders such as vitiligo and premature hair greying [152, 219]. For instance, it has been shown that the topical application of purified placental sphingolipid induced an enhanced MITF expression and its target genes *via* the stimulation of p38 stress-signalling pathway that restored follicular melanin-producing melanocytes and hair re-pigmentation *in vivo* in C57BL/6J mouse model of age-onset hair greying [152].

Based on these observations, it appears to be important to more precisely establish the molecular mechanisms associated with the anti-aging effects induced by calorie restriction, sirtuin activators and resveratrol on skin. Particularly, it will be essential to estimate their actions on the regenerative capacity and survival of skin-resident stem/progenitor cells and the maintenance of skin homeostasis *versus* their systemic toxicity and tumorigenic potential at long term. The optimization of the administration mode and therapeutic doses of these anti-aging agents seems also to be necessary before their use in safe conditions in human beings.

### Novel cell-replacement and gene therapies

The *in vivo* stimulation of endogenous skin-resident adult stem/progenitor cells or use of *ex vivo* expanded adult stem/progenitor cells or their differentiated progenies represent promising strategies for replacing dysfunctional or lost skin aged cells or obtaining physiologic tissue-engineered human skin equivalents for skin grafting after intense injuries [2, 38–43, 78, 135, 220]. Several therapeutic strategies have been investigated for skin reconstructive surgery after intractable and severe wounds such as burns, deep skin injuries, infections and decubitus ulcers, including cell grafting with diverse biocompatible artificial materials and/or skin fragments [39, 40]. For instance, the use of skin stem/progenitor cells and their progenies engineering for expressing human TERT or telomere RNA component (TR) represents a therapy of great interest for treating the chronological or premature aging disorders such as Werner progeroid syndrome, Fanconi anaemia and chronic dysplastic anaemia which

are associated with the defects in TERT and/or TR leading a replicative senescence of skin cells [38, 163]. Consistently, it has been reported that the bulge-resident ESCs in telomerase-deficient mice showed an impaired proliferative capacity that led to a decreased skin and hair growth [163]. The telomerase re-introduction in these mice, however, reversed the functional defects associated with a decreased telomerase activity and telomere shortening and restored the long-term survival of mice without increase skin tumorigenesis [163].

In addition, several data have revealed the beneficial effects to incorporate the dermal cells or BM-derived stem/progenitor cells for cell-replacement therapy and skin tissue engineering [39, 41, 78, 139]. The incorporation of dermal sheath cells (DSCs) in skin equivalents that can secrete diverse cytokines including insulin-growth factor-binding protein-2 (IGFBP-2), has been shown to enhance the number of putative p63-expressing KSCs, and improve the epidermal regenerative capacity [78]. Moreover, the use of cultured autologous BM-derived mesenchymal cells plus artificial dermis composite graft may also improve mixed chimerism and vascularized skin graft survival, and thereby promote the recovery of diverse types of intractable dermatopathies and prevent graft rejection [39]. Similarly, the inclusion of *ex vivo* expanded circulating EPCs from an autologous source onto tissue-engineered human skin substitutes has also been shown to result in the formation of functional microvessels that improved the perfusion and survival of bioengineered tissues [40].

### Novel cancer therapies targeting skin cancer stem/progenitor cells and their microenvironment

Since the skin stem/progenitor cells appear to provide critical functions in BCCs, SCCs and melanoma initiation, invasion and metastasis at distant sites, treatment resistance and disease relapse, several anti-cancer strategies have been designed to eradicate the total cancer cell mass including skin cancer-initiating cells and their differentiated progenies. Among the potential chemopreventive and therapeutic approaches, there are the use of natural dietary components, specific inhibitors of telomerase, EGFR, hedgehog, Wnt/ $\beta$ -catenin, Notch, Nodal/Cripto, hyaluronan (HA)/CD44 and/or SDF-1/CXCR4 signalling cascades and histone deacetylase (HDAC)-inhibitors as well as the molecular targeting of anti-apoptotic factors such as Bcl-2 and ABC multidrug transporters (Fig. 3) [1, 83, 192, 202, 221–226]. The results of *in vitro* and *in vivo* studies have revealed a beneficial effect to target these deregulated signalling elements, alone or in combination therapies, to inhibit the growth, survival, invasion and/or metastasis of skin cancer cells (Fig. 3) [173, 175, 203, 227–231]. Based on the observations that the telomerase re-activation may contribute to cell immortalization and cancer development, including skin cancers, several anti-telomerase strategies have been designed to inhibit the telomerase activity in cancer cells, and prevent the tumour development [170, 223, 232–236]. Moreover, the natural dietary component, resveratrol has been shown to act as a potent chemopreventive and chemotherapeutic agent against the

chemically- and UVB-induced non-melanoma development by activating the apoptotic pathways of cell death in mice models of skin carcinogenesis [35, 36]. Numerous studies have also revealed the beneficial effects to target the hedgehog signalling pathway to inhibit the growth and induce the apoptosis of BCC cells without major toxicity on normal cells [47, 192, 224, 237–239]. Among the hedgehog signalling inhibitor, there are SMO antagonists including a small molecule CUR61414 and the plant-derived steroidal alkaloid, cyclopamine or modulators of sterol levels including  $3\beta$ -hydroxysteroid (pro-)vitamin D3 and cholesterol synthesis inhibitors, statins (HMG-CoA reductase inhibitors) [47, 192, 224, 237–240]. Of particular interest, the data from a phase 1 clinical trial have also revealed that the treatment of BCC patients with a hedgehog inhibitor, may reverse the growth of the tumours in certain patients [241, 242]. Moreover, the inhibition of EGFR and Notch1 signalling using specific EGFR tyrosine kinase activity inhibitor, AG1478 and  $\gamma$ -secretase inhibitor, DAPT (N-[N-(3, 5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester) has also been reported to synergistically induce a substantial rate of apoptotic death of SCC cells [82].

In the case of melanoma, it has been shown that the use of dominant-negative EGFR, inhibitor of tyrosine kinase activity (TKI) of EGFR (gefitinib, AG1478 or PD153035) or KIT (imatinib mesylate) as well as the blockade of Wnt5/Frizzled/protein kinase C, Notch and Nodal/Cripto-1 cascades induced the cytotoxic effect on melanoma cells [203, 206, 210, 231, 243–246]. The targeting of HA/CD44 tumorigenic pathway using mAb or soluble CD44 protein form also diminished the human melanoma growth and/or incidence of metastases *in vitro* and in mice models *in vivo* (Fig. 3) [230, 247–249]. The pharmacologic suppression of MITF transcription factor expression, which provides critical role in the regulation of differentiation and survival of melanocytic cell lineage through a topical application of HDAC inhibitor also reduced the skin pigmentation and melanoma formation in mouse xenografts derived melanoma cells *in vivo* [250]. Importantly, the molecular targeting of sonic hedgehog SHH/PTCH/GLI by using smoothed co-receptor inhibitor, cyclopamine also prevented the growth, recurrence, and metastasis of melanoma xenografts in mice *in vivo* suggesting that the melanoma-initiating cells may be eliminated following this treatment type [226]. In this same way, the down-regulation of CD133 pentaspan transmembrane glycoprotein (prominin-1) in FEMX-I melanoma cells by short hairpin RNAs was also accompanied by a reduction of cell growth, cell motility, and capacity of cancer cells to form spheroids *in vitro* [65]. Moreover, the use of mAb directed against epitope 1 (AC133 and W6B3C1) or epitope 2 (AC141) of the CD133 protein, induced the cytotoxic effects in FEMX-I cells *in vitro* and markedly decreased their metastatic capacity in mice model *in vivo* (Fig. 3) [65]. The data from microarray analysis have also revealed that several gene products coding for Wnt inhibitors were up-regulated in the CD133 down-regulated cells [65]. These observations suggest then that the signalling pathway initiated through the CD133 stem cell-like marker can also contribute to malignant phenotype of melanoma stem cells in cooperation with Wnt cascade, and thereby constitute a potential molecular therapeutic target to eradicate the melanoma-initiating

cells. Similarly, it has been reported that the use of a mAb directed against ABCB5 efflux pump also inhibited the tumour growth of CD133<sup>+</sup>/ABCB5<sup>+</sup> melanoma stem cell-derived xenografts *in vivo* [63]. The ABCB5 blockade in the CD133<sup>+</sup>/ABCB5<sup>+</sup> melanoma stem cell subpopulation was accompanied by an intracellular doxorubicin accumulation that significantly restored drug sensitivity [58]. Additional characterization of the dose-responses and selectivity of these mAbs on melanoma stem/progenitor cells *versus* the normal adult stem cells expressing CD133<sup>+</sup>/ABCB5<sup>+</sup> *in vitro* and animal models *in vivo* is however required before their potential testing in clinical settings in safe conditions.

Other potential therapeutic strategies against invasive, metastatic and/or relapsed melanomas also include the immunotherapy-based melanoma vaccines, chemoimmunotherapies with immunosuppressive agents such as interferon- $\alpha$ , gene therapy and anti-angiogenic therapy by using thalidomine, angiostatin and/or endostatin, without or plus a stem cell-based transplant [112, 221, 227, 251–255]. More particularly, it has been reported that the melanoma stem cells, including CD133<sup>+</sup> clonogenic melanoma cells, express a significant level of cancer testis antigens (CTAs) suggesting that they could be efficiently targeted *in vivo* by CTA-directed immunotherapy [253, 255]. A therapeutic regimen consisting to 5-fluorocytosine plus engineered neural stem cells (NSCs) expressing cytosine deaminase, which acts as a pro-drug activating enzyme, also resulted in a significant reduction in the tumour border in animal models with established melanoma brain metastasis *in vivo* [254].

## Conclusions and future directions

Altogether, these recent advancements made in the field of skin-resident adult stem/progenitor cell biology have provided important information on the deregulated signalling cascades that may contribute to induce their dysfunctions, loss or malignant transformation during the chronological and accelerated aging and their pathological consequences. Additional studies are however necessary to more precisely establish the differentially-expressed gene products in these immature cells *versus* their differentiated progenies as well as the changes in their microenvironment occurring during human aging process. These future investigations should shed light onto intrinsic and extrinsic factors that may contribute to the progressive decline in regenerative capacity or loss of skin-resident adult stem/progenitor cells, and thereby help to develop new anti-aging strategies for preventing or delaying the onset of age-related skin disorders in human beings.

Further identification of specific biomarkers and drug targets in skin cancer stem/progenitor cells is also essential to more precisely establish their hierarchical organization and phenotypic features associated with the skin tumour development and progression from localized cancers in invasive and metastatic stages. In this regard, the establishment of specific functions of CD133

protein in normal bulge stem/progenitor cells and melanoma stem/progenitor cells is of immense interest to identify the co-partners that could be selectively targeted in highly tumorigenic and metastatic melanoma stem cells and without toxicity on normal CD133<sup>+</sup> regenerative stem/progenitor cells found in skin and other tissues. These additional studies should lead to identification of novel drug targets for eradicating skin tumour- and metastasis-initiating cells, and thereby improving the clinical treatments against aggressive and recurrent skin cancers, and more

particularly metastatic melanoma, which remain lethal with current therapeutic regimen options.

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