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Melanocyte stem cells in skin diseases and their potential in cell-based therapy

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Summary. Melanocytes have a complex function and play an important role in a variety of regulatory mechanisms in the human system. Melanocyte stem cells (MelSCs) serve as a reservoir to replenish the melanocytes by regenerating new ones, and they are capable of self-renewal and differentiation to maintain their homeostasis, repair, and regeneration in tissues. The numerical decrease and functional impairment of MelSCs may be closely related to the development and treatment response of many skin diseases. However, the current knowledge about MelSCs mainly comes from studies in mice, and little is known about human MelSC markers; especially, their markers are still unclear or lack consensus. This leads to uncertainty in clinical findings, which further limits our comprehensive understanding of pigmentary disorders and also hinders the progress of new treatments. Thus, in this review article, combined with our previous and current work, we summarize and update the recent advances in MelSC research, including the molecular markers of human MelSCs and their niche, as well as the association of MelSCs with skin diseases, including vitiligo, hair greying, and melanoma. Due to the limited tools available to explore the identified characteristics of human MelSCs, pluripotent stem cells can provide a new research model for further study, especially combined with CRISPR/Cas9 technology. The visualization of human MelSCs' development and differentiation can help to identify their molecular characteristics and understand their cellular fate dynamically, which will allow us not only to further explore their roles in associated diseases, but also to achieve MelSC-based cellular therapy.

Key words: Melanocyte stem cells, Pluripotent stem cells, Niche, CRISPR/Cas9, Stem-cell based therapy

Introduction

Human melanocytes are found mainly in the epidermis and hair follicles of the skin. Their basic function is to produce melanin granules, which protect the skin from ultraviolet light. In addition, melanocytes are "stress receptors" in the epidermis, providing molecular regulatory signals to a number of systems in the body. They not only have neuroendocrine functions, producing neurotransmitters, but also produce a range of growth factors involved in the regulation of immune responses (Tsatmali et al., 2002). Thus, melanocytes have a complex function and play an important role in a variety of regulatory mechanisms in the human system.

The idea that melanocytes originate from the neural crest has been widely accepted (Le Douarin et al., 2004; Ernfors, 2010). Recently, Cohen and her colleagues implanted neural crest stem cells which were derived from human embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs) into mouse

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Abbreviations. MelSCs, melanocyte stem cells; HFSCs, hair follicle stem cells; PSCs, pluripotent stem cells; iPSCs, induced pluripotent stem cells; ESCs, embryonic stem cells; NB-UVB, Narrow Bound Ultra Violet B Light; ORS, outer root sheath; LPP, lower permanent portion; DPs, dermal papilla cells; HF, hair follicle.

embryos and found that these cells produced functional melanocytes in chimeric mice after birth, further confirming this theory of origin (Cohen et al., 2016). Melanoblasts are the progenitors of melanocytes (Colombo et al., 2012), which migrate from the neural crest to the epidermis and the hair follicles during development (Lee and Fisher., 2014) and then are segregated into distinct populations during morphogenesis. One population will differentiate into mature melanocytes, which are immediately responsible for the pigmentation of hair or skin during the first hair cycle. The other population resides in the hair follicle bulge region, referred to as MelSCs. As the reserve source of melanocytes, melanocyte stem cells (MelSCs) are capable of self-renewal and differentiation to maintain their homeostasis, repair, and regeneration in tissues (Nishimura et al., 2002). They are normally quiescent, but when the hair cycle progresses from telogen to anagen, the majority of them are activated to divide, resulting in transient amplifying cells and their subsequent differentiation into functional melanocytes (Mull et al., 2015). MelSCs are not only responsible for pigmented hair regrowth under physiological conditions but are also involved in the repigmentation following various stresses and injuries, such as UV or ionizing radiation (Inomata et al., 2009; Ueno et al., 2014), wound injury (Chou et al., 2013; Yuriguchi et al., 2016), etc. In addition, a number of studies have shown that the numerical decrease and functional impairment of MelSCs may be closely related to the development of vitiligo (Seleit et al., 2014), melanoma (Moon et al., 2017), and hair greying (Nishimura et al., 2005) and may also be involved in the process of repigmentation after treatment.

However, the current understanding of human MelSCs is not as full as that in mice, and their molecular characteristics, stem cell niche, and signaling pathway, which affect their maintenance and differentiation, are vague. As a result, how they are involved in the development and regression of these diseases remains unclear, which limits our comprehensive understanding of the pathogenesis of these pigment-associated disorders and further hinders the progress of new treatments. In this review, we summarize the characteristics and microenvironment, including cell-cell interactions, extracellular matrix proteins, and critical signal pathways, of MelSCs, especially human ones. The differences in molecular markers of MelSCs between humans and mice are also discussed. In addition, the recent progress in the roles of MelSCs in skin diseases such as vitiligo, hair greying, and melanoma and potential therapeutic targets are also deeply related to this subject. Finally, a new model using human pluripotent stem cells (PSCs) is proposed for further study, which can help to better understand the development and unknown features of human MelSCs and to further explore the associated disorders.

Progress on MelSCs

Biomarkers

MelSCs are located in the lower permanent portion (LPP) of the hair follicle and are prompted to proliferate and differentiate into mature melanocytes during the anagen phase. As the hair follicle cycle progresses, differentiated melanocytes gradually undergo apoptosis, while MelSCs are retained (Nishimura, 2011). MelSCs have unique molecular characteristics; the common ones are discussed below.

DCT

DCT plays an important role in melanin synthesis, and it was found to be expressed in the hair bulb and also in the bulge region (Nishimura et al., 2002; Mak et al., 2006). By dynamic tracking, Dct-LacZ-expressing cells in the mouse LPP region were found to be self-renewing and multiply differentiated (Nishimura et al., 2002). Furthermore, LPP follicles containing Dct-LacZ-expressing cells were shown to provide melanin progenitor cells after transplantation. All these results suggest that Dct-labeled cells have stem cell characteristics (Nishimura et al., 2002).

To specifically demonstrate that Dct marks this MelSC population, Harris and his colleagues used the Tyr::CreERT2 mouse model and assessed the reconstituted cells by β-galactosidase staining. $LacZ^+$ cells were observed in the hair bulb. To further challenge their repopulation ability, they used plucking to eliminate all differentiated melanocytes, leaving only MelSCs to replenish newly generated hairs. Indeed, 7 days after initiating a new hair cycle (7 days after plucking), they observed $LacZ^+$ cells retained in the hair bulge. This confirmed that these $LacZ^+$ bulge cells were indeed MelSCs. They further analyzed these $LacZ^+$ MelSCs with immunolabeling and confirmed that almost all (97%) expressed *Dct*, which confirms *Dct* as a positive marker of MelSCs (Harris et al., 2013).

In humans, whether Dct could be used as a marker for MelSCs remains controversial. Nishimura and her colleagues found that melanocytes in human hair follicles do not express Dct (Nishimura, 2011). By contrast, Iman Seleit and her colleagues labeled melanocytes and the whole melanocyte lineage (including progenitor/stem cells) with HMB-45 and DCT, respectively, and used this difference to account for the presence of MelSCs at vitiligo lesions (Seleit et al., 2014). In addition, Goldstein and his colleagues found the presence of c-KIT^+ DCT^+ melanoblasts and c-KIT^- DCT^+ MelSCs in human hair follicles through dynamic observation of skin lesions in vitiligo patients (Goldstein et al., 2015). Another study also proved the existence of DCT^+ MelSCs in the follicular bulge of vitiligo patients (Birlea et al., 2017b).

MITF

MITF is a master transcriptional regulator of melanocyte development (Steingrímsson et al., 2004; Levy et al., 2006). Loss of function due to *Mitf* deletion or mutation results in melanocyte deficiency and pigmentation defects (Yasumoto et al., 2002). *Mitf* promotes the expression of many melanogenesis-related genes such as *Tyr* and *Pax3* (Qiu et al., 2015). In addition, *Mitf* can enhance the role of WNT/β-catenin signaling in the proliferation and differentiation of MelSCs through a feedback mechanism (Kubic et al., 2008). Although *Mitf* plays the above important role, it was found that mouse MelSCs (in pelage hair follicles at anagen V) did not express *Mitf* (Nishimura, 2011).

In humans, germline mutations in *MITF* can lead to Waardenburg syndrome or Tietz syndrome, characterized by a lack of pigmentation and deafness (Praetorius et al., 2013). Premature hair greying is observed in vitiligo patients carrying *MITF* mutations (Nishimura, 2011), and these examples from human genetics suggest that *MITF* is important for maintaining the human stem cell pool. Nishimura and her colleagues analyzed the distribution of melanoblasts in aging human hair follicles and found that MITF+ immature melanoblasts were abundant in follicles from 20- to 30-year-old subjects in the outer root sheath (ORS) around the bulge area (Nishimura et al., 2005), similar to previously described "amelanotic melanocytes" (Hu et al., 1957; Horikawa et al., 1996). However, they were absent from most hair follicles of 70- to 90-year-old subjects. These cells have been suggested to be a reservoir population for differentiated melanocytes and exhibit a very similar morphology to MelSCs in mice (Horikawa et al., 1996). Therefore, those groups of less differentiated and $MITF^{low}$ cells located in the LPP of human hair follicles were identified as human MelSCs (Nishimura et al., 2005).

In addition, immunohistochemistry for $MITF^{+}$ was performed on normal skin sections, and immunohistochemical analysis revealed that human MelSCs were characterized as $MITF^{+}$ cells existing in the bulge of hair follicles (Yamada et al., 2014). Recently, $MITF^{+}TYRP1^{-}$ MelSCs were also identified using the human PSCs differentiation system, and they were also found to be localized in the bulge region after transplantation into immunodeficient mice (Liu et al., 2019, 2020), also supporting MITF expression in human MelSCs.

PAX3

The ability of Pax3 to maintain the "high stemness" of melanocytes in the bulge region of mouse hair follicles has been demonstrated. In the regulation of melanogenesis, *Pax3*, which has a higher affinity for the *Dct* enhancer, maintains the quiescence of MelSCs by competing with *Mitf* for the binding of the enhancer responsible for the expression of *Dct* (Kubic et al., 2008) and can therefore replace *MITF* when its concentration is equal or higher (Lang et al., 2005). Studies have shown that mouse MelSCs express Pax3 (Lang et al., 2005; Osawa et al., 2005). Using $Pax3^{-}GFP^{+}$ mice, *Pax3* was detected in the epidermis and hair follicles at all stages of melanocyte differentiation, including the MelSC stage (Osawa et al., 2005). Joshi and his colleagues established a Tet-off double transgenic mouse model, Dct-H2B GFP, and found that the Pax3-expressing cells in the rondelles and secondary hair germ of resting hair follicles also expressed the MelSC markers Dct and c-Kit (Joshi et al., 2018), further confirming these findings. In humans, whether *Pax3* is expressed in MelSCs is not clear yet.

SOX10

SOX genes play key roles in embryonic development and are major determinants of stem cell behavior, regulating cell fate decisions, and maintaining cellular identity (Sarkar and Hochedlinger, 2013). In the proliferation, differentiation, and survival of MelSCs, *Sox10* acts synergistically with Pax3 to activate the transcription of Mitf, which in turn acts as a master regulator of MelSCs by controlling the expression of various melanocyte genes such as *Dct, Tyrp1,* and *Tyr* (Bondurand et al., 2000; Britsch et al., 2001). As mentioned above, Dct was identified as a marker for mouse MelSCs. Using antibodies, Harris observed that nearly all Dct^{+} cells within hairs co-express Sox10 regardless of time point, location, or differentiation status (Harris et al., 2013). In addition, Shakhova and his colleagues made use of the Dct::LacZ transgenic mouse line expressing LacZ driven by Dct promoter, which allows genetic tracking of MelSCs in the hair follicle. Sox10 expression was detected in X-Gal-positive MelSCs located in the bulge region, further proving the expression of Sox10 in MelSCs (Shakhova et al., 2015). However, whether Sox10 is expressed in human MelSCs needs further study.

c-KIT

Mutations in *c-Kit*, or administration of c-Kit-neutralizing antibodies during mouse embryogenesis, prevent normal melanocyte development, such as melanoblasts proliferation, migration, and survival (Nishikawa-Torikai et al., 2011). Previous studies identified immature $Dct\text{-}lacZ^{+}/c\text{-}Kit^{low}$ melanoblasts located in the bulge area of hair follicles as MelSCs (Nishimura et al., 2002; Nishimura et al., 2005). When neonatal mice were treated with anti-c-Kit-blocking antibodies, only a fraction of the MelSC population survived, and the residual $c\text{-}Kit^{+}$ MelSCs were able to reconstitute the melanocyte lineage in the hair follicle and epidermis (Nishimura et al., 2002). The findings of Nishikawa-Torikai also prove this conclusion. By using $Dct^{tm1(Cre)Bee}$/CAG-CAT-GFP mice, only $c\text{-}Kit^{low}$ side $scatter^{low}$ cells proliferated and differentiated into

pigmented cells. In culture, these cells maintain their capacity to differentiate and can reconstitute an MelSC system in HFs, which further proves that MelSCs express low levels of c-Kit (Botchkareva et al., 2001; Nishikawa-Torikai et al., 2011). Also, Inomata found that c-Kit expression with no melanin content is a good marker for MelSCs in non-aged mice (Inomata et al., 2009). Recently, Tandukar found that, in telogen HFs, all the GFP$^+$ MelSCs expressed c-Kit, with higher expression observed in the secondary hair germ region, which further proves c-Kit expression in MelSCs (Tandukar et al., 2021).

In humans, c-Kit expression was found in human amelanotic melanocytes (at mid-anagen), which further identified human MelSCs (Nishimura, 2011). Besides, in the isolation and propagation of highly purified populations of primary human limbal melanocytes, it was demonstrated that human limbal MelSCs show a higher level of c-Kit expression than limbal melanocytes (Polisetti et al., 2020), which is consistent with the conclusion of Nishimura.

PMEL17

Mouse *Pmel17* is one of the murine melanosomal genes (Baxter and Pavan, 2003); it promotes melanin biosynthesis in melanocytes by forming functional amyloid fibers (Fowler et al., 2006). Using transgenic mice carrying the Dct-lacZ reporter gene, the expression of MelSCs was analyzed, and Pmel17 was found expressed in MelSCs (Osawa et al., 2005). Similarly, Joshi and his colleagues found significant levels of Pmel17 expression in telogen-phase MelSCs (Joshi et al., 2018), which further proved the existence of Pmel17$^+$ MelSCs.

Previously, immunohistochemical studies on anagen human hair follicles have suggested that the melanocyte progenitor population located in the bulge area was PMEL17$^+$ (Narisawa et al., 1997). In addition, PMEL17 expression was found in human amelanotic melanocytes (at mid-anagen), further confirming PMEL17 expression in human MelSCs (Nishimura, 2011).

FRIZZLED4

As one of the melanocyte lineage markers and the receptors of the WNT pathway, Frizzled4 participates in many skin diseases (Pawar and Rao, 2018). Real-time PCR revealed that *Frizzled4* was expressed higher in the bulge area than other areas. In addition, FACS analysis showed that populations of Frizzled4$^+$ cells required a longer culture period to differentiate into mature melanocytes than c-Kit$^+$ cells (previously known as melanoblasts). Furthermore, upregulation of mRNA expressions of melanocyte markers *Dct, Tyr,* and *Tyrp1* was observed in Frizzled4$^+$ cells during differentiation (Yamada et al., 2010). These results suggested that Frizzled4$^+$ cells were more immature than melanoblasts, therefore raising the possibility that Frizzled4$^+$ cells are MelSCs. Recently, Qiu and his colleagues further proved the existence of FRIZZLED4$^+$ MelSCs (Qiu et al., 2019). By immunostaining, FRIZZLED4 expression was found in both human and mouse hair follicles, especially in the ORS (Guo et al., 2019). Immunohistochemistry of FRIZZLED4 in human normal skin revealed that human MelSCs were characterized as FRIZZLED4$^+$ cells existing in the bulge of hair follicles (Yamada et al., 2014).

CD34

CD34 is a well-known molecular marker of stem cells (Amoh et al., 2005). A recent study revealed a functional heterogeneity of MelSC subtypes, and CD34 defines a subpopulation of MelSCs with unique regenerative characteristics (Joshi et al., 2019). Using Dct-H2BGFP mice, bulge and secondary hair germ MelSCs were separated using CD34. CD34$^-$ MelSCs expressed higher melanocyte genes, representing MelSCs in a more advanced state of melanocyte differentiation, and therefore, CD34 can serve as a negative molecular marker for MelSCs with higher melanocyte differentiation potential. In contrast, CD34$^+$ MelSCs are not fully committed to terminal melanocyte differentiation and exhibit a broader range of neural crest stem cell characteristics, and they are able to selectively differentiate into glial and myelinated neurons. Using the same mouse model, Tandukar further demonstrated the superior differentiation ability of CD34$^-$ MelSCs by higher expression of melanogenic markers *Tyr* (71±1%), *Tyrp1* (68±4%), and *Mitf* (75±7%) (Tandukar et al., 2021). Thus, CD34 could be used as a negative marker for MelSCs when combined with the above positive-expressed ones.

Collectively, the current knowledge of MelSCs comes mainly from studies in mice, and the human data are quite limited. In addition, the molecular characteristics of MelSCs are inconsistent or even contradictory across species and studies.

Niche and critical signal of MelSCs

The niche, also called the stem cell microenvironment, maintains stem cell pools and promotes their quiescence. It regulates stem cells through direct contact with neighboring cells, the extracellular matrix, and signaling molecules. The niche of MelSCs was identified in the LPP of the hair follicle, and it plays a major role in regulating the quiescence, differentiation, proliferation, and survival of MelSCs (Nishimura et al., 2002). In this part, the niche and critical signal pathways for MelSCs' maintenance are discussed (Fig. 1).

Adjacent cells and extracellular matrix

In addition to MelSCs, the hair follicle LPP contains at least another stem cell population, namely hair follicle

stem cells (HFSCs) (Morris et al., 2004). Because these two stem cell populations reside in the same region anatomically, it makes sense that there would be some interaction between these cells. MelSCs undergo activation and differentiation with HFSCs to regenerate pigmented hair. The interaction between these two stem cell populations was demonstrated within the niche using a double transgenic Tyr-CreER^{T2} and β-catenin$^{fl(ex3)/+}$ mice model (Rabbani et al., 2011). It investigated how activation of the WNT signal in HFSCs affects MelSCs behavior and concluded that stabilization of β-catenin in HFSCs exogenously affects MelSCs. HFSCs in the hair follicle niche also regulate themselves and MelSCs by producing extracellular matrix proteins, such as a semi-bridging granule transmembrane collagen protein called collagen XVII (also known as COL17A1) (Tanimura et al., 2011). This protein is attached to the underlying basement membrane, and its absence leads to reduced cell anchoring, hair greying and loss, and shrinkage of the hair follicle (Powell et al., 2005). The distribution and morphology of MelSCs were first examined in COL17A1-deficient mice by using Dct-lacZ mice, in which it was demonstrated that MelSC maintenance is defective, which is caused by defects in the HFSC population that forms the main supportive cells surrounding MelSCs (Tanimura et al., 2011).

Furthermore, dermal papilla (DPs) cells also exert some influence on MelSCs. Mc1r activity is the key point to decide the type of melanin (Slominski et al., 2005; Qiu et al., 2019), and it can be inhibited by DP-derived agouti, which promotes the production of pheomelanin (Ollmann et al., 1998; Jackson et al., 2007). This suggests that, as a signal center, DP not only regulates various hair follicle behaviors (Lei et al., 2017) but also plays an important role in regulating hair pigmentation (Qiu et al., 2019).

The hair follicle bulge is located in a complex microscopic environment, and extrafollicular signals also have a regulatory role in MelSCs (Chueh et al., 2013). Other tissues surrounding the hair follicle, such as neurons (Katayama et al., 2006, Zhang et al., 2020), dermis/adipose tissue (Plikus et al., 2008), or systemic hormones (Chueh et al., 2013), can influence the behavior of stem cells. Under stress, the sympathetic nervous system is activated to induce the fight or flight response by secreting norepinephrine from peripheral axon terminals (Ulrich-Lai and Herman, 2009). Recently, Zhang and his colleagues identified that norepinephrine secreted by sympathetic nerve endings signals acts on the MelSCs, leading to hair greying (Zhang et al., 2020).

Signal pathways

The maintenance of MelSCs is critical because their loss leads to a failure of follicular melanocyte

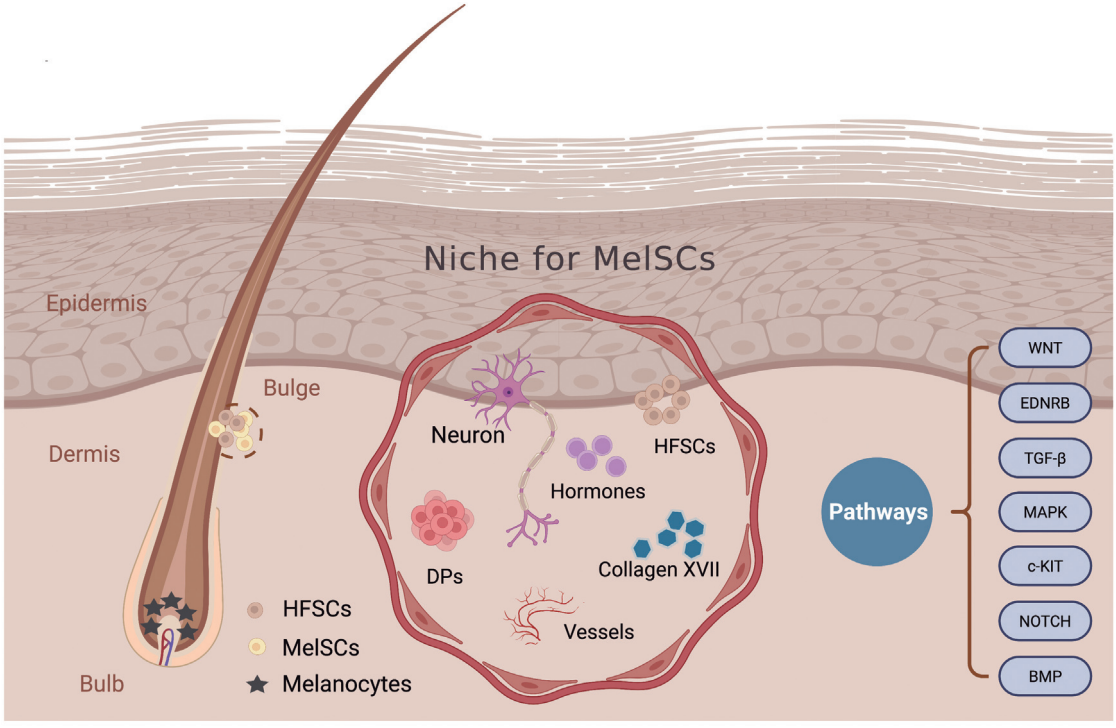

Fig. 1. Niche and critical signal pathways in MelSCs' maintenance. MelSCs, melanocyte stem cells; HFSCs, hair follicle stem cells; DPs, dermal papilla cells. Created with BioRender.com.

regeneration (Nishimura et al., 2005; Steingrímsson et al., 2005). In addition to the cells surrounding MelSCs and the extracellular matrix, multiple signal pathways participate in the maintenance of stationary MelSCs and their differentiation (Osawa et al., 2005).

WNT/β-catenin is one of the most classic pathways which influence the maintenance of MelSCs' quiescence by affecting the molecular balance of *Pax3, Sox10,* and *Mitf* in MelSCs (Kubic et al., 2008; Adam et al., 2018). Inhibition of the WNT signal helps to maintain the niche homeostasis of MelSCs, while deactivation of the typical WNT signal induces endothelin-1 expression in HFSCs, which promotes MelSCs' differentiation (Rabbani et al., 2011). EDNRB was also identified to play an important role in MelSCs' maintenance and proliferation in hair follicles (Takeo et al., 2016). In addition, NOTCH (Callahan and Egan, 2004; Aubin-Houzelstein et al., 2008), TGF-β (Nishimura et al., 2010), SCF/c-KIT (Aoki et al., 2011; O'Reilly-Pol and Johnson, 2013), and MAPK (Lee and Fisher, 2014) also participate in the maintenance of MelSCs.

The roles of MelSCs in pigmentation-related diseases

Defects or deficiencies in the number and function of melanocytes and/or MelSCs can lead to pigmentation abnormalities, resulting in the development of diseases such as vitiligo and hair greying. Melanoma, one of the most aggressive human cancers, is also thought to be linked to MelSCs. Despite our understanding of MelSCs as described above, how they are involved in the development and regression of these diseases remains unclear. In the following, progress in understanding the relevance of MelSCs to these diseases is discussed, which is also summarized in Table 1.

Vitiligo

Vitiligo is the most common human pigmentation disorder, affecting approximately 0.1% to 2.0% of the world's population (Howitz et al., 1977; Boisseau-Garsaud et al., 2000; Alikhan et al., 2011), and its pathogenesis is not fully understood. It is characterized by the progressive destruction of mature melanocytes in the epidermis and manifests clinically as progressive depigmentation of the skin and mucous membranes (Le Poole et al., 1993; Schallreuter et al., 2008; Ezzedine et al., 2012). The contrast between normally pigmented and lesioned skin causes a range of psychological and social stigmatization in patients (Birlea et al., 2017b). Several mechanisms have been proposed to cause melanocyte destruction, including autoimmunity (Le Poole and Luiten, 2008), auto-cytotoxic/metabolic mechanisms (Dell'Anna et al., 2007), and impaired melanocyte migration and/or proliferation (Gauthier et al., 2003). Genetic susceptibility also plays an important role in the development of vitiligo (Spritz, 2007). However, none of these processes is sufficient to fully explain the mechanisms of disease, and all proposed mechanisms are not mutually exclusive. Therefore, convergent theories have been developed combining biochemical, environmental, and immunological events within a permissive genetic environment (Taïeb, 2012).

Rare involvement of MelSCs in the development of vitiligo

Existing hypotheses of vitiligo pathogenesis generally suggest that a $CD8^+$ T cell attack on epidermal melanocytes is a key event (Spritz and Anderson, 2017; Rashighi and Harris., 2017; Roberts et al., 2020). Thus, are MelSCs involved upstream of melanocytes? Does normal pigment loss result from depletion of MelSCs or progenitor cells? (Bellei et al., 2013) To confirm this conjecture, Iman Seleit and her colleagues examined the skin of 50 vitiligo patients by immunohistochemical staining for HMB-45. They found that melanocyte progenitors/MelSCs were detectable in 54% of the interfollicular epidermis and in 63.3% of the follicular epidermis in areas of lesioned skin (Seleit et al., 2014). It is indicated that vitiligo skin lesions may not arise due to the depletion of MelSCs. Another study examined the expression of multiple melanocyte lineage markers, including *Dct, c-Kit,* and *Tyr*, in the depigmented areas of vitiligo patients (Birlea et al., 2017b). Tyr^+ mature melanocytes were not detected in the follicular bulge, the funiculus, or the interfollicular epidermis in the depigmented white patches; however, these areas were rich in MelSCs to be activated ($c\text{-}KIT^-DCT^+$) and melanoblasts ($c\text{-}KIT^+DCT^+$). The numbers were similar to those found in areas of vitiligo lesions after NB-UVB

Table 1. Correlation between MelSCs and pigmentation-related diseases.

Diseases	Base model	MelSCs Marker	Correlation	References
Vitiligo	Patients	Dct	The presence of MelSCs in interfollicular epidermis of vitiligo may provide an additional reservoir needed for repigmentation	Seleit et al., 2014
Vitiligo	Mouse	Frizzled4	UVB irradiation induces activation of WNT7a, stimulating differentiation of MelSCs, leading to repigmentation of vitiligo	Yamada et al., 2013
Hair greying	Mouse	Dct	Hair greying is caused by a self-maintenance defect in MelSCs	Nishimura et al., 2005
Hair greying	Mouse	Dct	Stress induces hair greying by depleting the MelSC pool	Zhang et al., 2020
Melanoma	Mouse	Tyr-CreER	MelSCs show melanoma-forming potential	Moon et al., 2017
Melanoma	Mouse	c-Kit	c-Kit+ MelSCs induce epidermal melanoma formation	Sun et al., 2019

treatment and in the skin of healthy controls. All these results suggested that the development of vitiligo may not be significantly associated with MelSC depletion and MelSCs are retained in vitiligo lesions.

Involvement of MelSCs in vitiligo repigmentation

Although MelSCs may not be directly involved in vitiligo development, several studies suggest that these cells may play an important role in the repigmentation process. Clinically, repigmentation in vitiligo occurs in different patterns, the most prevalent of which is perifollicular (hair follicle-centered) (Parsad et al., 2004; Yang et al., 2010). Notably, clinical observations have shown that hair follicles can provide epidermal melanocytes during vitiligo recovery (Ortonne et al., 1979; Cui et al., 1991) and are a major source of repigmentation.

Human hair follicle cultures revealed the presence of MelSCs with significant proliferative and further melanization potential in hair follicles (Arrunátegui et al., 1994). Using the double transgenic K14-steel factor (SLF)/Dct-lacZ mouse model (Tobin et al., 1995), it was observed that Dct-lacZ$^+$ progenitor melanocytes in the bulge could migrate upwards to replenish epidermal melanocytes (Nishimura et al., 2002). It is also suggested that the bulge area of the hair follicle is a reservoir of MelSCs that replenish melanocytes in the epidermal layer of the skin, and it plays an important role in the restoration of vitiligo (Nishimura et al., 2002). To determine whether MelSCs in hair follicles produce epidermal melanocytes, Chou and his colleagues created excisional wounds on the dorsal skin of mice and monitored the movement of melanocytes using Pax3-labeled MelSCs as label-retaining cells, confirming that MelSCs can move from the follicle to the epidermis after injury (Chou et al., 2013).

Although there is no conclusive evidence that repigmentation of vitiligo is dominated by MelSCs, several clinical studies have suggested this possibility. As early as the 1950s, it was found that ultraviolet light exposure caused the appearance of pigmented islands in areas of vitiligo depigmentation, with hair follicle cores containing melanocytes with large cell bodies (Montagna and Chase, 1956). Subsequently, the same phenomenon was found in vitiligo patients treated with herbal remedies (Ortonne et al., 1980). The presence of DOPA-positive melanocytes in the epidermis was detected after transplantation of the patient's autologous hair follicles into areas of depigmented leukoplakia (Cui et al., 1991), suggesting the presence of melanocyte progenitors in the hair follicle pool. In addition, human hair follicle outer root sheath cell suspension, which acts as a MelSC source (Vanscheidt and Hunziker, 2009; Mohanty et al., 2011; Singh et al., 2013), has been shown to be effective to treat stable vitiligo (Mohanty et al., 2011; Shah et al., 2016) and it can repigment 65.7% of vitiligo patches after transplantation (Mohanty et al., 2011). Recently, using vitiligo model mice, Han and his colleagues found that clinically acceptable micro-injuries induce the hair follicle MelSCs to migrate upwards to the interfollicular epidermis, activating and giving rise to melanocytes to repopulate the vitiligo lesion (Han et al., 2022). These studies demonstrate the role of MelSCs in vitiligo repigmentation and also suggest their potential for the treatment of depigmentation disorders. However, the exact molecular mechanisms whereby MelSCs are involved in vitiligo repigmentation are not yet fully understood.

Repigmentation induced by UV stimulation of MelSCs

Birlea and her colleagues found that UV light and/or drugs can effectively activate melanocyte progenitor in hair follicles and epidermis. Of these, NB-UVB is the most potent stimulus for inducing epidermal melanocyte regeneration (Birlea et al., 2017a; Barbulescu et al., 2020). Yamada previously demonstrated that UVB induces the differentiation of MelSCs into melanoblasts in hair follicles. To investigate the involvement of MelSCs in UVB-induced repigmentation in hair follicles, the mRNA expression level of *Frizzled4*, the MelSC marker, was analyzed. They found that *Frizzled4* expression was transiently decreased in hair follicles by UVB irradiation on days 1-3; conversely, Frizzled4$^-$/Tyrp1$^+$ cells (melanoblasts) emerged on days 1-3 (Yamada et al., 2013). They demonstrated that UVB irradiation induces activation of WNT7a and stimulates differentiation and upward migration of MelSCs in the hair bulge, leading to repigmentation around the hair follicles of vitiligo (Yamada et al., 2013). In addition, Nishimura suggested that MelSCs are maintained in a stationary state via TGF-β and that UVB irradiation leads to a reduction in TGF-β signaling (Nishimura et al., 2010), which may help to lower the threshold for MelSCs' activation, thereby facilitating repigmentation. To further understand the molecular changes that occur in the hair follicle and interfollicular epidermis during NB-UVB treatment, melanocyte progenitors were selectively extracted by laser capture microdissection techniques from the follicular bulge of untreated and NB-UVB-treated vitiligo skin, respectively. Whole transcript RNA sequencing found that melanocyte progenitors in the follicular bulge of vitiligo skin were controlled by RHO-GTPase, KCTD10, and CTNNB1 signals during NB-UVB treatment (Goldstein et al., 2021).

In summary, MelSCs may not be directly involved in the development of vitiligo, but during treatment such as UVB, they can be activated by different pathways, thus differentiating, maturing, and participating in the repigmentation process of vitiligo.

Hair greying

Aging-related hair greying

One of the hallmarks of aging is the progressive

decline in the function of tissue-specific stem cells involved in tissue maintenance and repair, and hair greying is one of the distinctive phenotypes of human aging. Approximately 50% of the human population will have approximately 50% of their hair turn grey by the time they are around 50 years old. Under physiological conditions, MelSCs and their progeny constitute the pigment system of the hair follicle. Several studies have suggested that age-related hair greying is attributable to changes in MelSCs. Loss of MelSCs and mature melanocytes has been found in the aging human bulge (Steingrímsson et al., 2005). Similar findings have been observed in the hair follicles of individuals of different ages, with higher numbers of MelSCs in follicles of subjects aged 20-30 years and reduced numbers of MelSCs in follicles of subjects aged 40-50 years (Nishimura et al., 2005).

Recently, Yu and her colleagues established a mouse model with skin aging which was induced by localized irradiation (Yu et al., 2021). They found that these mice showed significant aging-like phenotypes such as baldness, which was due to HFSC exhaustion, and this process was associated with miR-31 upregulation. In addition to hair loss, striking hair greying was also found in this model. Although it was previously found that HFSCs, rather than MelSCs, are the primary target for radiation (Aoki et al., 2013), considering that HFSC and MelSCs share the same niche, it is conceivable that changes in the miR-31 pathway in HFSCs can indirectly modulate MelSCs and induce hair greying.

Hair greying is also associated with age-related defects in MelSC maintenance. DNA damage caused by genotoxic stressors such as ionizing radiation triggers MelSC differentiation, and MelSC depletion triggers "irreversible" greying through differentiation or apoptosis (Rabbani et al., 2011; Yamada et al., 2013), a classic example being UV light. The possible mechanism is as follows: WNT7a secreted by UV-irradiated epidermal keratinocytes causes sustained activation of β-catenin signal in MelSCs, the latter promoting the maturation of MelSCs' differentiation, failure, and premature greying of hair (Rabbani et al., 2011; Yamada et al., 2013). Premature hair greying is also commonly seen in a number of progeria syndromes, such as Werner syndrome and ataxia telangiectasia (Hasty et al., 2003). As these diseases have been characterized by genomic instability, genomic stress caused by endogenous or exogenous sources might be responsible for MelSC changes with aging (Nishimura, 2011).

Although it has recently been proposed that hair greying is initially caused by dysfunction of the major hair follicle pigmentary unit in the hair-growing stroma, away from the bulge and independent of MelSC activity, the results do not exclude the simultaneous independent accumulation over time of (ultimately irreversible) damage to MelSCs in the bulge stem cell niche (O'Sullivan et al., 2021).

Stress-related hair greying

Different types of stress, including genotoxic stress and genetic and epigenetic alterations, can abrogate MelSCs' renewal or maintenance, leading to stress greying. A recent study investigating how acute stress leads to hair greying significantly advanced these findings (Zhang et al., 2020). Using mouse models, it was found that MelSCs undergo loss under stressful conditions independent of immune attack or adrenal stress hormones. Instead, stress activates sympathetic nerves innervating the MelSCs' niche, leading to a sudden release of the neurotransmitter norepinephrine, and free norepinephrine directly binds to β2-adrenergic receptors on MelSCs, resulting in the activation, differentiation, and ectopic migration of approximately 50% of MelSCs in the niche (Zhang et al., 2020; Yardman-Frank et al., 2021), in contrast to the 6% activation of stem cells in the conventional hair cycle, an over-proliferation that rapidly leads to MelSC depletion and hair greying in the subsequent hair follicle cycle. It was also found that acute stress-induced greying could be prevented by blocking the sympathetic release of norepinephrine, preventing norepinephrine from binding to MelSCs, or inhibiting melanocyte proliferation.

To test whether this adrenergic signal might similarly induce ectopic pigmentation in MelSCs *in vitro*, Rachmin and her colleagues isolated human hair follicles and treated them with norepinephrine (Rachmin et al., 2021). Ectopic pigmentation of MelSCs with increased expression of TRP-1 and DCT was detected using brightfield microscopy. It was demonstrated that human hair follicles can respond to sympathetic neurotransmitter stress signals, which can induce premature differentiation of MelSCs and amelanotic cells.

Other reason-related hair greying

By using multiple animal models, it was further shown that depletion of MelSCs in the augmentation or reduction of their function leads to the production of hair greying (Nishimura et al., 2005; Harris et al., 2018). Nishimura and her colleagues used Dct-lacZ transgenic mice to demonstrate that hair greying is caused by a self-maintenance defect (Nishimura et al., 2005). They found that *Bcl2* deficiency leads to selective apoptosis of MelSCs in the niche when they enter a dormant state, significantly accelerating the process of physiological greying. Hemimorphic mutations in *Mitf* trigger a more progressive greying process (Lerner et al., 1986), which is associated with progressive ectopic differentiation of MelSCs within the niche (Nishimura et al., 2005; Harris et al., 2018). In addition, *Sox10* knockdown would lead to loss of MelSCs and differentiated melanocytes, while overexpression of *Sox10* leads to premature differentiation, which also leads to loss of MelSCs, finally resulting in hair greying (Harris et al., 2013).

Therapeutic targets for hair greying

Although the role of MelSCs in hair greying has been demonstrated in multiple studies or aging-related settings, effective and predictable therapeutic strategies to prevent or reverse human hair greying have not yet been developed. In addition, the question of whether the process of age-related hair greying is reversible remains unsolved.

SCF, HGF, and END3 are found to be capable of promoting the proliferation and differentiation of melanoblasts or melanocytes, which are potent factors in preventing hair greying (Qiu et al., 2019). Among these factors, SCF seems to be more effective (Endou et al., 2014). Sustained expression of SCF in epidermal keratin-forming cells was able to rescue the number of damaged MelSCs in *Bcl2*$^{-}$deficient mice, thereby preventing hair greying (Mak et al., 2006). It has been shown that CXCL12 regulates the differentiation of MelSCs as well as their proper localization, and maintaining MelSCs by regulating CXCL12 expression levels in the rondel region may also be an effective means of preventing hair greying (Yamada et al., 2019).

Recently, a potential combination therapy RT1640, which consists of cyclosporine A (CsA), minoxidil, and RT175, was identified to have the ability to combat the process of hair greying (Anderson et al., 2021). CsA is an immunosuppressive calcium-regulated phosphatase inhibitor that regulates hair growth by inducing and prolonging growth hormone, enhancing hair follicle stem cell activity, and blocking the ability of dermal papillae to initiate degeneration (Horsley et al., 2008; Hawkshaw et al., 2018;). Minoxidil promotes the transition from resting to anagen phase, increases the percentage of larger hair follicles, and reduces hair loss through its multifaceted effects on dermal papillae, subcutaneous adipose tissue, and the perifollicular vascular system (Messenger and Rundegren, 2004; Suchonwanit et al., 2019). RT175 is a derivative of the immunosuppressant FK506, which has been shown to have immunomodulatory and regenerative properties, acting through independent mechanisms. Using a mouse model of hair greying, RT1640 therapy not only statistically increased the mean size of the MelSC pool in hair but also activated hair growth faster than the potent regeneration inducer cyclosporine A (Anderson et al., 2021). This effect appears to rely on the combined activity of the three drugs to activate both hair growth and MelSC.

Interestingly, hair repigmentation was also observed in a clinical study which used anti-programmed cell death 1 (anti-PD-1) therapies to treat non-small cell lung cancer (Rivera et al., 2017). Fourteen patients receiving anti-PD-1 therapy presented hair repigmentation during follow-up. However, the mechanism is still unclear. A recent study indicated that migrating melanocytes may occasionally repopulate the depleted MelSCs' niche and may produce pigmented hair (Yardman-Frank et al., 2021). Therefore, all the above suggest that hair greying can be reversible, and it would be intriguing to develop new therapies to treat hair greying.

Melanoma

Melanoma is an aggressive cancer with a high degree of malignancy, and its development involves a variety of factors, including genetics and the environment. Although most melanomas arise ab initio, some melanomas evolve from pre-existing pigmented nevi (Weatherhead et al., 2007). It has been suggested that melanocytes in the basal layer of the epidermis gradually transform, undergoing a progressive metamorphosis from nevus to the radial growth phase, to the vertical growth phase, and finally to metastatic malignant melanoma (Bevona et al., 2003).

MelSC-originated melanoma

Numerous clinical studies have identified genetic alterations in human cutaneous melanoma with *BRAF* (BrafV600E and other mutations, ~60%) and *PTEN* (deletions, ~25%) (Hodis et al., 2012; Vultur et al., 2014). Therefore, investigators constructed the Tyr-CreER; LSL-BrafV600E; Ptenflox/flox (TBP) mouse model for preclinical studies of melanoma (Dankort et al., 2009; Damsky et al., 2011). Although Moon and colleagues demonstrated the potential for melanoma formation derived from hair follicle MelSCs in the Braf CA/$^{+}$ (Braf V600E/$^{+}$) and Ptenfl/fl (Tyr-CreER:Braf:Pten) mouse models (Moon et al., 2017), Kohler et al. did not find their tumor-forming capacity using the same model (Köhler et al., 2017). Because Tyr-CreER can label both MelSCs located in hair follicles and melanocytes in the epidermis (Harris et al., 2013), while melanomas form primarily in the dermis of these mouse models (Dankort et al., 2009), it is difficult to determine the origin of melanoma. Qi Sun used *c-Kit* to label MelSCs in mouse hair follicles and found that *c-Kit*$^{+}$ MelSCs were able to induce epidermal melanoma formation in a combined oncogenic BrafV600E-induced and Pten-deficient mouse model, thus demonstrating that MelSCs are the true source of melanomas that will expand and subsequently invade the dermis. Furthermore, interception of normal WNT and endothelin niche signals during the early stages of hair growth would promote malignant transformation of MelSCs during melanoma induction (Sun et al., 2019).

UVB radiation associated with MelSC-originated melanoma

UVB radiation is the main environmental physical carcinogen associated with melanoma. Decades of epidemiological studies have linked UVB radiation to the development of malignant melanoma (Moan et al., 2008). Among these, UVB exposure is one of the major risk factors for cutaneous melanoma (Sample and He, 2018).

UVB not only accelerates epidermal proliferation and skin inflammation but also induces MelSC migration from the hair follicle to the interfollicular epidermis in both mice and humans (Chou et al., 2013). By optimizing the UVB dose to effectively induce hair follicle MelSCs to migrate to the interfollicular epidermis without initiating anagen induction, Chou and his colleagues found that the shaved dorsal skin of TBP mice could form flesh-visible melanomas within 12-16 days of exposure to UVB; they also found that local UVB exposure can initiate melanoma production through activation of MelSCs and uniform translocation throughout the skin. Although MelSCs are stationary after the initial transition to the anagen phase, UVB is sufficient to induce MelSC activation and migration at any stage of the hair cycle to initiate melanoma production (Moon et al., 2017).

Hmga2, a chromatin remodeling transcriptional regulator, has long been recognized as an oncogene in many cancers, including melanoma (Raskin et al., 2013), and it is widely involved in cancer progression and metastasis (Morishita et al., 2013). It was found that function loss of Hmga2 in skin was able to significantly inhibit melanoma formation by suppressing UVB-mediated MelSC activation and translocation, thus providing a novel strategy for the prevention of melanoma induced by MelSCs (Moon et al., 2017).

Unaddressed issues

Although great progress has been made surrounding MelSCs, including their characteristics, niche, critical signaling pathway, and potential roles in skin diseases, what is known about human MelSCs is extremely limited, and the current evidence is mainly from mouse research. Since mouse skin is quite different from human skin in both embryonic development and anatomy, the results of the mouse studies are not fully applicable to humans, and there are still many questions waiting to be answered. Much of the direct evidence on human MelSCs comes from human skin sampling, but this is limited by the inability to observe the dynamics of development, as occurs in mouse models. Other methods to define MelSCs, such as colony-forming capacity, are neither reliable nor unique because neighboring stem/progenitor cells such as Nestin$^+$ cells and some Schwann cells also have this capacity (Dupin et al., 2003, Van Raamsdonk and Deo, 2013). Therefore, the specific markers of human MelSCs are still absent. How exactly to define MelSCs, especially human MelSCs, is a matter of debate and has become a major obstacle to understanding their role in the related diseases. A new research model which can address these issues is urgently needed.

Future trends in modeling with pluripotent stem cells

Human PSCs, including ESCs and iPSCs, possess the capability of self-renewal and multiple-directional differentiation (Takahashi and Yamanaka, 2006; Okita et al., 2007). Due to these advantages, they have strong potentials in disease models, drug screening, and cellular therapy.

Practical model of MelSCs

In addition to the generation of mature cells, human PSCs (hPSCs) can also mimic embryonic development and generate somatic stem cells in specific lineages, which can be used to study their development and maturing. Systems have been successfully established for neural crest stem cells (Menendez et al., 2013), vascular endothelial and hematopoietic progenitors (Lange et al., 2016), epicardial cells (Bao et al., 2017), cardiac progenitors (Zhang et al., 2019), and skeletal muscle progenitors (Sato, 2020). Furthermore, combining patient-derived iPSCs and gene editing techniques, specific disease models can be constructed, which will help to better understand the underlying mechanisms and also to propose individualized and precise treatments (Dimos et al., 2008; Soldner et al., 2009).

Currently, several studies have reported the successful generation of melanocytes from human PSCs (Nissan et al., 2011; Kunisada et al., 2014; Liu et al., 2019, 2020). These induced melanocytes expressed melanocyte markers such as MITF, TYR, SILV, TYRP1, etc., and their global gene expression profile showed a high degree of similarity to human primary melanocytes. In addition, melanosome production was also identified by electron microscopy. Therefore, hPSCs are expected to provide a new model to study the development and maturation of human melanocytes. However, there are very few studies involving MelSCs.

Recently, Cohen and his colleagues implanted human ESC- and iPSC-derived neural crest stem cells into mouse embryos, confirming the origination of melanocytes from the neural crest (Cohen et al., 2016). This study raises the possibility of exploring the development process of MelSCs dynamically using human PSCs. Moreover, Hosaka identified a unique, self-renewing cell lineage, melanocyte progenitor cells (MPCs), which are derived from human PSCs (Hosaka et al., 2019). These MPCs exhibit a morphology distinct from melanocytes and lack the melanosomal structure or melanocyte-specific marker genes MITF, TYR, and SOX10. By activating the canonical WNT pathway with a small amount of GSK3β inhibitor, these cells change into typical dendritic mature melanocytes containing abundant melanin granules. Our team established a three-dimensional suspension system with high differentiation efficiency in which human PSCs can be efficiently differentiated into both MelSCs and mature melanocytes (Liu et al., 2019, 2020). These mixed melanocytes integrated more efficiently than human primary melanocytes in immunodeficient mice and maintained long-term *in vivo* function after transplantation. We found that MITF$^+$PAX3$^+$TYRP1$^+$

mature melanocytes integrated well into the basal epidermis and hair bulb of mice and could deliver melanin granules normally to mouse keratinocytes to generate pigmented hairs. In addition, $MITF^{+}PAX3^{+}$ $TYRP1^{-}$ MelSCs integrated into the bulge and sub-bulge region of reconstituted hair follicles and supported their long-term function. More importantly, these integration sites are the physiological distribution areas of mature melanocytes and MelSCs in both humans and mice. Furthermore, gene dynamics studies showed that early markers during melanocyte development such as *Mitf, Pax3,* and *Sox10* were detected as early as day 7 of differentiation, while mature markers like *Tyr* and *Tyrp1* showed a significant increase until day 21 (Liu et al., 2020). This dynamic pattern of stepwise expression and progressive increase of specific markers not only confirms their mutual regulation but also suggests that the differentiation system of human PSCs *in vitro* may provide a useful model for studying the development of MelSCs.

Exploration of melanocyte origin and development

To achieve dynamic tracing of the stem cell, targeting genes with fluorescent protein reporter genes is the most common method. CRISPR/Cas9 is the fastest-developing targeted gene editing technology, and it has been widely used in the genome of many organisms (Hsu et al., 2014; Ma et al., 2014; Khan et al., 2016). This technology has many advantages, such as a high rate of gene modification, diverse gene regulation, simultaneous knockout of multiple target loci, precise targeting, and inexpensiveness. Therefore, the CRISPR/Cas9 technique plays a significant role in visualization of cell fate, mechanism exploration of known mutations, and screening for pathetic genes (Zhou et al., 2021). The combination of CRISPR/Cas9 and human PSCs through gene knock-out or knock-in enables observation of phenotypic changes and, potentially, the identification of disease targets for clinical research and therapy.

Using CRISPR/Cas9, the visual dynamic tracking, separation, and purification of cardiac and skeletal muscle stem cells were successfully achieved by stably knocking in the reporter gene in human PSCs (Zhang et al., 2019). Based on the unique role of PAX7 and MYF5 in muscle stem cell and early skeletal muscle lineage specification, Wu and his colleagues generated a PAX7/MYF5 double-reporter human ESC line using CRISPR/Cas9-mediated homologous recombination and then identified CD10 and CD24 as specific markers for skeletal muscle stem cells using a surface marker screen (Wu et al., 2018). Similarly, by using CRISPR/Cas9, a double reporter for TBX5 and NKX2-5 in human PSC was established to delineate cardiac lineages and help to isolate the lineage-specific subpopulations successfully (Zhang et al., 2019). The above research shows that the combination of human PSCs and CRISPR/Cas9 can provide a good dynamic observation model for the development, characterization, and fate tracking of somatic stem cells and provide reference value for the further research of MelSCs.

In addition, the development and differentiation of MelSCs can also be explored using single-cell sequencing and bioinformatics to characterize each cell subpopulation at different time points of human PSC differentiation. This will also help to identify the characteristic of human MelSCs.

Stem cell-based therapy

As the most representative cellular therapy using stem cells, hematopoietic stem cell transplantation (HSCT) has been widely used clinically. Both autologous and allogeneic HSCT provide a revolutionary way to prolong patients' life with blood diseases such as multiple myeloma (Koniarczyk et al., 2017) and lymphoma (Lee and Hong, 2020), sickle cell disease (Tanhehco and Bhatia, 2019), and autoimmune diseases such as multiple sclerosis (Mancardi and Saccardi., 2008; Moore et al., 2019), systemic sclerosis (van Laar et al., 2014), and Crohn's disease (Ruiz et al., 2015). However, unlike hematopoietic stem cells, somatic stem cells are not available for the treatment of other types of diseases.

Due to the advantages of self-renewal and multidirectional differentiation, human PSCs can provide large numbers of target cells for cellular therapy and address the above issue. Compared to ESCs, human PSCs have fewer moral and ethical issues, making them a new avenue in cellular regeneration and transplantation therapy. In addition, they could provide a truly individualized therapy which is customized for patients. Human PCS-based therapy is also widely studied in various diseases involved in different organs or tissues, such as Parkinson's disease. Autologous iPSC-derived dopaminergic neurons (DA) were transplanted into the brains of cynomolgus monkeys with MPTP-induced injury, and it was found that the motor disorder was significantly improved (Hallett et al., 2015). Furthermore, human PSC-derived DA progenitor cells have been shown to survive, mature, and function well as midbrain DA neurons in a primate Parkinson's disease model (Kikuchi et al., 2017; Zhou et al., 2018). With these encouraging preclinical results, clinical trials using human ESC/iPSC-derived cell products to treat PD have been started in Australia (nct02452723), China (nct03119636), and Japan (jma-iia00384; umin 000033564). In addition to Parkinson's disease, clinical trials of cellular therapies using human PSC in Japan have been ongoing for treating 7 types of diseases, including macular degeneration (Yamanaka, 2013), corneal disorder, retinitis pigmentosa, etc. Clinical trials for thrombocytopenia, spinal cord injury, and ovarian clear cell carcinoma have also been approved.

Thus, these studies show us the great potential of human PSCs in regenerative medicine and also provide reference for the application of PSC-derived MelSCs

and melanocytes to treat depigmentation diseases such as vitiligo and hair greying in the future.

Conclusion

Although MelSCs are involved in many pigment-related disorders, such as vitiligo, hair greying, and melanoma, many questions remain unsolved. The lack of uniformly accepted human MelSC markers leads to uncertainty in clinical findings, which further limits our deeper understanding of pigmentary disorders and also hinders the progress of new treatments. Due to the current limitation, hPSC combined with CRISPR/Cas9 technology may help to achieve the visualization of MelSCs and explore their fate dynamically. This new perspective will not only help to explore important signal pathways and key molecules during melanocyte lineage development; reveal the fate of melanocytes in organ development, tissue formation, and cell differentiation; and understand the pathogenesis and regulatory pathways of diseases such as vitiligo and melanoma, but it also provides a purification and enrichment method for MelSCs, which may provide a source for stem cell-based therapy.

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