Multipotent skin-derived precursors: adult neural crest-related precursors with therapeutic potential

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We previously made the surprising finding that cultures of multipotent precursors can be grown from the dermis of neonatal and adult mammalian skin. These skin-derived precursors (SKPs) display multi-lineage differentiation potential, producing both neural and mesodermal progeny in vitro, and are an apparently novel precursor cell type that is distinct from other known precursors within the skin. In this review, we begin by placing these findings within the context of the rapidly evolving stem cell field. We then describe our recent efforts focused on understanding the developmental biology of SKPs, discussing the idea that SKPs are neural crest-related precursors that (i) migrate into the skin during embryogenesis, (ii) persist within a specific dermal niche, and (iii) play a key role in the normal physiology, and potentially pathology, of the skin. We conclude by highlighting some of the therapeutic implications and unresolved questions raised by these studies.

Keywords: stem cells; dermal papilla; hair follicles; spinal cord injury; cancer; plasticity

1. INTRODUCTION

A number of studies published in the past 7 years have challenged the traditional view that stem cells present in somatic tissues are restricted to producing that tissue’s cell types. Neural stem cells isolated from the embryonic or adult central nervous system (CNS) have been reported to differentiate into muscle (Galli et al. 2000; Tsai & McKay 2000; Bani-Yaghoub et al. 2004), blood (Bjornson et al. 2000), embryonic or adult central nervous system (CNS) tissue’s cell types. Neural stem cells isolated from the embryonic neural crest (NC) and blood vessel-associated haematopoietic and endothelial precursors. Moreover, differentiation of skin-derived precursors from skin.

2. ISOLATION AND DIFFERENTIATION OF NOVEL MULTIPOTENT PRECURSORS FROM SKIN

Skin represents a complex and highly regenerative tissue containing a relatively broad stem cell repertoire, including resident epidermal and mesenchymal stem cells (MSCs), migratory melanocytic stem cells derived from the embryonic neural crest (NC) and blood vessel-associated haematopoietic and endothelial precursors. The idea that skin might provide an accessible, potentially autologous source of neural precursors (NPCs) was first suggested by the finding that Merkel cells, a type of sensory receptor with characteristics of neurons, could be generated within the skin of rodents (Nurse et al. 1984). On the basis of these findings, we hypothesized that skin contained a precursor cell with neurogenic capacity and used methods similar to those used to culture CNS neural stem cells (Reynolds & Weiss 1992; Reynolds et al. 1992) in an attempt to isolate these precursors. When skin cells of neonatal and adult rodents were dissociated to single cells and grown in suspension culture in the presence of the mitogens fibroblast growth factor-2 (FGF2) and epidermal growth factor (EGF), floating spheres of proliferating cells were generated (Toma et al. 2001). Importantly, clonally derived colonies were able to produce this same complement of cell types, confirming the multipotency of these cultured precursors from skin.

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from epidermal stem cells (Toma et al 2001; Fernandes et al 2004). In particular, they were antigenically distinct from MSCs and, when grown under the same conditions, the MSCs grew adherently while SKPs grew as floating spheres (Toma et al 2001). SKPs were derived from the dermis and did not apparently produce keratinocytes, distinguishing them from MSCs and hematopoietic stem cells, nor other mesodermal cell types known to be resident in skin or subcutaneous muscle. Non-specific cytokeratin staining also illuminates the subcutaneous muscle layer. (ii) Enzymatically and mechanically dissociated mouse skin. (iii) Growth of SKP spheres in suspension cultures when dissociated skin cells are exposed to the mitogens FGF2 and EGF. Under appropriate conditions, SKP spheres differentiate into neurons that express βIII tubulin, glial cells that express S100β, smooth muscle cells that express smooth muscle SMA and adipocytes containing characteristic intracellular lipid droplets. FGF2, fibroblast growth factor-2; EGF, epidermal growth factor; SMA, smooth muscle actin.

A direct comparison of these precursors with other types of stem cells and precursors known to be resident in skin indicated that skin-derived precursors (SKPs) were a distinct population (Toma et al 2001; Fernandes et al 2004). In particular, they were antigenically distinct from MSCs and, when grown under the same conditions, the MSCs grew adherently while SKPs grew as floating spheres (Toma et al 2001). SKPs were derived from the dermis and did not apparently produce keratinocytes, distinguishing them from epidermal stem cells (Toma et al 2001). Finally, SKPs neither express c-kit, a marker of melanocytic stem cells and haematopoietic stem cells, nor other melanocyte stem cell markers such as dct (Fernandes et al 2004). We also compared SKPs directly to CNS neural stem cells cultured as neurospheres and again found significant differences; while both CNS stem cells and SKPs expressed nestin, SKPs also expressed a number of proteins, including fibronectin, that were not expressed by CNS neural stem cells (Fernandes et al 2004). In addition, while both CNS stem cells and SKPs could generate one mesodermal cell type, smooth muscle cells, only SKPs were able to generate adipocytes (Toma et al 2001). Thus, on the basis of these findings, we proposed that SKPs were novel multipotent precursors resident in the mammalian dermis (figure 1).

3. SKPs: WHAT, WHERE AND WHY?
The notion of multi-lineage adult stem cell plasticity has been challenged by a number of findings in recent years, including demonstrations of cell fusion by bone marrow-derived cells (Alvarez-Dolado et al 2003; Vassilopoulos et al 2003; Wang et al 2003; Nygren et al 2004), an inability to repeat earlier reports of plasticity (Morshead et al 2002; Vallieres & Sawchenko 2003; Wehner et al 2003) and the production of non-functional cell types (Lu et al 2004; Neuhuber et al 2004). These findings raised several fundamental questions regarding the biological properties and in vivo relevance of cultured adult stem cells, which we have attempted to address with regards to SKPs. What is their developmental origin? Are SKPs an endogenous precursor cell? Where are SKPs located in vivo and what are their biological roles?

(a) SKPs exhibit properties of embryonic neural crest precursors
The capacity of SKPs to generate both neural and mesodermal progeny provided our first clue to their potential developmental origin. In particular, one embryonic precursor population with a similar developmental potential is NC stem cells. The embryonic NC is a subpopulation of neuroectodermally derived cells that undergoes an epithelial–mesenchymal transformation and subsequently exits from the dorsal neural tube. These embryonic NC cells consist of a transient population of multipotent and fate-biased precursors that ultimately generate a wide variety of non-CNS cell types. Among the clearly identified derivatives of NC cells are the entire peripheral (sensory, autonomic and enteric) nervous system, catecholaminergic adrenal cells, melanocytes, Merkel cells, and, at the cranial levels, the facial dermis, cartilage, bone, adipocytes and meninges (Halata et al 1990; Couly et al 1992; Le Douarin & Kalcheim 1999; Etchevers et al 2001; Jiang et al 2002a; Le Douarin & Dupin 2003). The NC also contributes a variety of mesodermal cell types outside the cranial regions, including adipocytes within parasympathetic ganglia of the gut (Le Lievre & Le Douarin 1975), peripheral nerve fibroblasts (Halata et al 1990; Joseph et al 2004)

Figure 1. Isolation and differentiation of skin-derived precursors. (a) Isolation of SKPs. (i) Fluorescence micrograph of postnatal mouse skin, with nuclei labelled by Hoechst (blue) and epithelial cells of the epidermis and hair follicles labelled with anti-cytokeratin (green). Non-specific cytokeratin staining also illuminates the subcutaneous muscle layer. (ii) Enzymatically and mechanically dissociated mouse skin. (iii) Growth of SKP spheres in suspension cultures when dissociated skin cells are exposed to the mitogens FGF2 and EGF. (b) Differentiation of SKP spheres. Under appropriate conditions, SKP spheres differentiate into neurons that express βIII tubulin, glial cells that express S100β, smooth muscle cells that express smooth muscle SMA and adipocytes containing characteristic intracellular lipid droplets. FGF2, fibroblast growth factor-2; EGF, epidermal growth factor; SMA, smooth muscle actin.

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that SKPs are NC-related precursors in the skin that

and the outflow tract of the heart (Fukiishi &
Morriss-Kay 1992; Serbedzija & McMahon 1997;
Jiang et al. 2000; Chan et al. 2004). Clonal analysis

of embryonic NC cells showed that migrating multipotent

NC precursors ultimately reach a variety of different

target tissues and, while they were traditionally thought
to terminally differentiate within these tissues, recent
evidence has raised the possibility that these NC
precursors persist in at least some adult tissues such

as the gut (Kruger et al. 2002). We therefore examined

the possibility that SKPs may be NC-derived pre-
cursors that are maintained into adulthood within the
dermis of the skin.

These studies revealed four lines of evidence supporting
this possibility (Fernandes et al. 2004).
First, a side-by-side comparison of gene expression in
SKPs and NC-containing embryonic neural tube
revealed that SKPs express a variety of NC-associated
transcription factors including Slug, Snail, Twist, Pax3
and Sox9. Moreover, SKPs also expressed SHOX2 and
Dermo-1, the transcription factors associated with the
mesenchymal capability of cranial NC cells. In
contrast, few of these transcription factors were
expressed by CNS neural stem cells. Second, pheno-
typic analysis of the neural cells differentiated from
SKPs revealed that they were largely peripheral
nervous system cell types including Schwann cells
and catecholaminergic neurons, cells that are derived
from the NC during normal development. Third, when
genetically tagged SKPs were transplanted into NC
migratory pathways in the embryonic chick, they were
able to specifically follow the host NC migratory
streams, ultimately ending up in peripheral sensory
and autonomic ganglia, peripheral nerves and the skin.
Fourth, when SKPs were derived from the facial skin of
Wnt1-cre;R26R compound transgenic mice, which selectively and indelibly express β-galactosidase in

in peripheral NC derivatives, SKP spheres were β-
galactosidase positive (Fernandes et al. 2004; figure 2).

Together these data strongly support the hypothesis that
SKPs are NC-related precursors in the skin that
retain multipotency into adulthood. These data also
indicate that SKPs generated from facial skin derive

from the NC embryonically. Do SKPs generated from
trunk skin share this embryonic origin? Unfortunately,

attempts to use the same lineage tracing approach to
answer this question were confounded by the partial
penetration of the Wnt1-cre;R26R β-galactosidase
transgene in back skin (Fernandes et al. 2004).
In particular, while only a fraction of SKPs isolated from
the back skin of these mice were β-galactosidase
positive, many known NC progeny, including some
melanocytes and Schwann cells, were also not
β-galactosidase positive in the back skin (Fernandes
et al. 2004), making such studies inconclusive. Thus, it
is formally possible that trunk-derived SKPs have all of
the properties of NC precursors, but derive from a
different, as-yet-uncharacterized population of NC-like
precursors in the embryo, and/or that they derive from
NC stem cells that do not express Wnt1.

Figure 2. SKPs from the facial skin of Wnt1-cre;R26R
compound transgenic mice. Spheres generated from the facial

skin of Wnt1-cre;R26R transgenic mice, which indelibly
express β-galactosidase in neural crest derivatives, react
positively with the β-galactosidase substrate X-gal.

(b) SKPs are endogenous precursors that arise

in skin during embryogenesis and persist into
adulthood

A key question that has emerged in the stem cell field
over the past several years is to what extent cultured
precursor cells reflect the properties of the endogenous
cells from which they are derived (Fernandes et al.
2004). This issue has arisen as a consequence of studies
showing (i) that biased progenitors can dedifferentiate
in culture to become multipotent (Kondo & Raff
2000), (ii) that cells with similarities to embryonic
stem cells can be derived upon long-term culture of, for
example, bone marrow cells (Jiang et al. 2002b,c), and
(iii) in the most remarkable example, that nuclei
isolated from even terminally differentiated cells like
olfactory receptor neurons can be used to clonally

generate an entire mouse (Eggan et al. 2004; Li et
al. 2004). Thus, while it has always been thought that
embryonic precursor cells like NC stem cells exhibit
broader potential in culture than in their more restrictive
in vivo environments, these new studies raise the
possibility that much more profound alterations can occur as a consequence of long-term culture.

Is the multipotential nature of SKPs a consequence
of such culture-induced alterations? Two lines of
evidence argue against this possibility. In one series of

studies, we asked whether skin contained a precursor
cell capable of generating neurons, a SKP-derived
cell type never found in skin, without any passing and

expansion as spheres. In these experiments, skin cells
that were plated under conditions used to analyse the
neurogenic potential of primary embryonic NC stem
cells (Stemple & Anderson 1992; Shah et al. 1996;
Morrison et al. 1999) were capable of generating cells
with the morphological and antigenic characteristics
of differentiated peripheral catecholaminergic neurons
(Fernandes et al. 2004). Thus, skin contains a NPC
cell that normally does not generate catecholaminergic

neurons in vivo, but that can do so when placed in the
appropriate environmental conditions in culture. In a
second series of studies, we cultured skin cells under
conditions where single cells gave rise to single, clonal
spheres. These primary clones had all of the charac-
teristics of SKPs, and when differentiated were multi-

potent. Thus, SKPs that had never been passaged had

the same properties as SKPs that had been expanded in
culture (Fernandes et al. 2004). Moreover, this clonal
sphere assay allowed us to determine when the

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endogenous SKPs first arrive in skin. These studies indicated that mouse SKPs could first be detected in the back skin at embryonic day 15 (E15), that their numbers are then relatively high during late embryogenesis, decrease to lower levels at birth and then are maintained into adulthood (Fernandes et al. 2004). Together, these studies support the idea that SKPs represent an endogenous embryonic NC-derived precursor cell that persists into adulthood, and whose full potential is normally restricted by its in vivo environment but is revealed in culture.

(c) One niche for SKPs is the dermal papillae of hair and whisker follicles

If SKPs are restricted by their skin environment from generating, for example, neurons, then what is their in vivo environment? Four lines of evidence indicate that one niche for SKPs is the dermal papillae of hair and whisker follicles (Fernandes et al. 2004). First, in situ hybridization for transcription factors expressed by SKPs (as well as embryonic NC stem cells) such as Slug, Snail and Twist revealed that the most robust expression of these markers in adult mouse skin occurred in dermal papillae of follicles during the anagen growth phase. Thus, follicle papillae cells express SKP markers. Second, SKPs express markers that are enriched in follicle dermal papillae, including nixin, Wnt5a and versican. Third, when dermal papillae are dissected from whisker follicles and cultured under SKP conditions, they generate floating spheres of cells with the characteristics of SKPs. Finally, lineage tracing with the Wnt1-Cre;R26R mice revealed that whisker follicle dermal papillae are entirely NC derived, and that even dermal papillae of the back skin hair follicles contain NC-derived cells. Thus, these findings support the idea that follicle dermal papillae are a niche for the endogenous NC-derived SKPs. However, this is probably not the only niche for SKPs in skin, since SKPs can be routinely derived from human foreskin (Toma et al. 2005; described below), a tissue that contains no hair follicles.

Interestingly, follicular dermal cells, including dermal papillae cells, have long been thought to be multipotent precursors that potentially play an important role in normal skin turnover and in wound healing (Jahoda & Reynolds 2001; Gharzi et al. 2003). In this regard, cultured follicular dermal papilla cells have been shown to generate mesodermal cell types, including adipocytes and osteoblasts (Jahoda et al. 2003), and to even contribute to the haematopoietic system (Lako et al. 2002). They are also known to be the key regulators of hair follicle initiation and the follicle growth cycle, something that is discussed below. Like SKPs, follicle papillary cells proliferate in response to FGF2 and have been described as growing in clusters (Jahoda & Oliver 1984). Finally, follicle dermal papillae first condense at about E15 in mouse back skin, at the same time-point when SKPs can first be isolated, and the cells that form these embryonic papillae are positive for both TrKC and the p75 neurotrophin receptor (Borchikarev et al. 1998, 1999), markers of embryonic NC precursors (Stemple & Anderson 1992; Luo et al. 2003). Thus, the convergence of findings for follicular papillary cells and SKPs strongly support the idea that NC-derived SKPs comprise at least a fraction of the cells within the follicle dermal papillae.

4. PROSPECTS FOR THERAPEUTIC APPLICATIONS OF SKPs

One of the most exciting aspects of the ongoing work on SKPs is the prospect that they will provide an accessible, multipotent human precursor cell for a variety of purposes. While the most obvious of these is their potential use as an accessible source of cells for transplantation, human SKPs could also provide accessible human precursors for discovery research, something discussed in more detail below. As a requisite to such studies, however, it was essential to derive methods for isolating and expanding human SKPs, and to demonstrate that, like the rodent SKPs, they represented an accessible, multipotent precursor cell population.

(a) Human SKPs are similar to rodent SKPs

The initial work indicating that isolation of human SKPs was feasible was performed using ‘scalp tags’ from the placement of the stereotaxic neurosurgical apparatus on patients ranging from 40 to 70 years of age; small clusters of nestin-positive, fibroconnectin-positive cells could be derived from these very small scalp biopsies (Toma et al. 2001), suggesting that human skin also contained a SKP-like cell. More recent work using other human skin sources has now verified that human SKPs can be isolated and expanded, and are very similar to their rodent counterparts in terms of their potential. In our own work, we have focused upon human foreskins from 0–2-year-old males, and using a protocol very similar to that used for the rodent cells, we can routinely isolate and expand human SKPs. These human SKPs express markers typical of rodent SKPs, including NC transcription factors, and can differentiate into both neural and mesodermal progeny. Importantly, clonal analysis demonstrated that a single human cell can give rise to cells of both embryonic lineages. In addition, analysis of expanded human SKPs demonstrated that they maintain their differentiative abilities for at least 1 year in culture, and that these long-term passaged human SKPs maintained a normal karyotype, at least as analysed by G-banding. With regard to their embryonic origin, it is difficult to definitively ascertain whether human SKPs represent NC-derived precursors that are laid down in skin during embryogenesis and persist post-natally, as we believe to be the case for rodent SKPs. However, a number of lines of evidence indicate that they may well be. First, human SKPs express transcription factors expressed by NC stem cells. Second, human foreskin contains a population of NPCs that can differentiate into neurons without any isolation, passaging or expansion. Third, human SKPs have a potential similar to NC precursors, generating mesodermal progeny such as adipocytes, and neural cell types that are peripheral in nature, such as Schwann cells and catecholaminergic neurons (Toma et al. 2003).
Two recent papers have provided independent evidence in support of the existence of SKP-like NPCs in human skin (Belicchi et al. 2004; Joannides et al. 2004). In one study, the dermis of skin biopsy samples from 41 to 77-year-old healthy adults were grown into SKP-like spheres using FGF2 and EGF, and then the isolated spheres were adherently expanded using a serum-based protocol. These expanded adherent cultures retained the ability to generate spheres expressing the NPC markers nestin and musashi when placed in SKP-like serum-free conditions, and clonal analysis revealed a neural/mesodermal bipotentiality. Spheres generated following serum-based expansion were able to produce neurons upon differentiation, and neurogenesis was enhanced in the presence of hippocampal astrocyte-conditioned medium (Joannides et al. 2004), as previously reported for CNS NPCs (Song et al. 2002).

In a second study, skin samples were obtained from 14-week aborted foetuses and from 12 to 65-year-old healthy volunteers. In this study, skin cells were first fluorescent activated cell sorted for the human AC133(+) fraction, which has previously been shown to contain the sphere-forming cells from multiple stem cell populations, including human neural stem cells (Uchida et al. 2000). Consistent with this notion, no sphere-forming activity was found in the AC133-negative population. Single AC133(+) cells grew into spheres that could be clonally passaged, expressed nestin, and could differentiate into neuronal cells. Following transplantation into the lateral ventricles of neonatal immune-compromised SCID mice, cells from the AC133(+) derived spheres displayed robust migration and some evidence of neuronal and glial differentiation (Belicchi et al. 2004). Thus, cells with the properties of SKPs have been isolated from human skin of differing ages and origins, supporting the notion that SKPs provide an accessible, autologous human stem cell population.

(b) Functionality of SKP-derived neural and mesodermal cells

For any precursor cell population to be used therapeutically, it is essential that rigorous functional assays be performed to definitively establish that they actually generate bona fide differentiated cell types. This is particularly true as there are experimental limitations to many of the assays that are commonly used. For example, some groups have found that bone marrow-derived cells can, under some conditions, undergo a rapid apparent neuronal transdifferentiation, but recent studies indicate that such 'neurons' are actually non-functional (Lu et al. 2004; Neuhuber et al. 2004). In addition, the apparent transition of bone marrow to neural, cardiomycocyte and hepatic lineages in vivo may be largely, if not completely, due to cell fusion (Alvarez-Dolado et al. 2003; Vassilopoulos et al. 2003; Wang et al. 2003; Nygren et al. 2004). Thus, it is essential to ensure the functionality of cell types generated from stem cells via a variety of assays with complementary strengths. In this regard, our approach has been to first pursue culture studies, looking for (i) appropriate morphological changes, (ii) selective induction and expression of cell type-specific genes, and (iii) stability of differentiated cellular phenotypes, and to then use both ex vivo and in vivo transplantation to assay functionality. We are currently pursuing such an approach to ascertain the validity and functionality of SKP-derived neural and mesodermal cell types, and will discuss our neural work in more detail here.

As mentioned previously, SKPs apparently generate peripheral neural progeny, consistent with a NC origin. Schwann cells are a peripheral glial cell type of therapeutic interest, owing to their ability to myelinate axons and to provide a conducive growth and regeneration environment for CNS axons (Richardson et al. 1980, 1984; David & Aguayo 1981; Xu 1995b, 1997, 1999; Pearse et al. 2004). To ask whether SKPs generate bona fide Schwann cells, we performed a series of culture and in vivo studies, all of which strongly support the conclusion that SKP-derived Schwann cells are ‘real’ (McKenzie et al. 2006). First, SKPs responded to extrinsic cues that have been shown to enhance the differentiation and the proliferation of Schwann cells from NC stem cells to produce cultures highly enriched in cells with the morphological and biochemical phenotype of Schwann cells; they generated parallel arrays of bipolar cells that expressed the Schwann cell markers S100β, glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), protein 0 (P0), peripheral myelin protein-22 (PMP22) and p75NTR (Fernandes et al. 2004). Importantly, they do not express the proteins characteristic of neurons or other mesodermal cell types. Second, when these SKP-derived Schwann cells were cultured in the presence of peripheral neural explants, they associated with axons, divided and expressed myelin proteins, while at the same time undergoing a morphological differentiation consistent with myelination. Third, when SKPs were transplanted into either the peripheral nerve or the CNS of shiverer mutant mice, which are genetically deficient in a key myelin protein, MBP (Rosenbluth 1980a,b), they myelinated axons, as shown by electron microscopy. Significantly, this myelination was also seen with human SKPs. Thus, by all criteria, including production of functional myelin, SKPs generate bona fide Schwann cells (McKenzie et al. 2006).

What about SKP-derived neurons? Differentiation of SKPs under neurogenic conditions has resulted in the production of cells that fulfil most, but not yet all, criteria for neuronal differentiation (Fernandes 2006). In culture, differentiating rodent SKPs undergo an apparent neuronal transdifferentiation, but recent studies indicate that such 'neurons' are actually non-functional (Lu et al. 2004; Neuhuber et al. 2004). Importantly, they do not express any non-neuronal protein that we have examined. These SKP-derived neurons further mature when they are transplanted into slice cultures derived from the hippocampus, as they express and maintain expression of the catecholaminergic neurotransmitter enzyme,
tyrosine hydroxylase, for at least five weeks, and begin to express neuronal voltage-gated calcium channels. Interestingly, the neurons differentiated from SKPs maintain their peripheral phenotype, even in this CNS environment. However, the gold standard for demonstrating functional neuronal differentiation is appropriate electrophysiological characteristics, including the generation of depolarization-induced action potentials, something that we are starting to assay now. In general, reaching this standard using multipotent stem cells has proven to be difficult, requiring long and somewhat complex differentiation protocols and the presence of astrocyte-associated factors. Nonetheless, this is a necessary demonstration that will provide the ultimate proof that a given stem cell-derived cell is a bona fide neuron.

(c) Potential clinical applications for human SKPs

We are currently exploring several potential therapeutically relevant uses for SKPs, including autologous cell-replacement strategies, the effects of genetic alterations on stem cell function and differentiation and the potential involvement of transformed SKP-like precursors in tumours of NC-derived tissues.

(i) Cell-replacement strategies

The ability to differentiate SKPs into functional cell types has potential therapeutic implications for autologous cell-replacement strategies. As described above, a particularly promising avenue is cell therapy using SKP-derived glial cells, as we have generated considerable data indicating that SKPs can be used to produce functional myelinating Schwann cells which have potent growth-promoting effects on injured CNS neurons, and have extensively been used to promote regeneration of CNS axons in animal spinal cord injury models (Richardson et al. 1980; Paino et al. 1994; Xu 1995a, b, 1997, 1999; Guest et al. 1997; Menei et al. 1998; Oudega et al. 2001; Bunge & Pearse 2003; Pearse et al. 2004; Fouad et al. 2005). Moreover, the ability of Schwann cells to myelinate may make them useful replacement cells for oligodendrocytes that degenerate following spinal cord injury (Crowe et al. 1997; Shuman et al. 1997; Li et al. 1999; Casha et al. 2001; Warden et al. 2002; Beattie et al. 2002) or as a consequence of multiple sclerosis (Brierley et al. 2001; Halfpenny et al. 2002; Bachelin et al. 2005).

Besides neural cell types, we have also found that SKP cultures can be directed to generate skeletogenic derivatives normally associated with cranial NC cells. Specifically, SKPs reproducibly generate characteristic nodules that express chondrocyte oligomeric matrix protein and type II collagen, synthesize chondrocytic proteoglycans and ultimately produce mineralized calcium deposits associated with osteoblast activity (K. Fernandes, J. Biernaskie, J.-F. Lavoie and F. Miller 2002, unpublished data). Thus, SKPs may be useful as a source of certain mesenchymal derivatives.

(ii) Mechanisms of disease development in genetic disorders

In addition to transplantation-based therapies, the availability of accessible, autologous NPCs also opens novel doors to exploring the mechanisms and potential treatments of genetically based neural disorders. SKPs derived from humans having neurodegenerative conditions may serve as useful tools for investigating the effects of specific genetic alterations on the essential properties of NPCs (i.e. survival, proliferation and differentiation) or, alternatively, on the characteristics and functions of cells differentiated from mutant NPCs. Such studies on the cell biological properties of genetically compromised precursors, neurons or glial cells could provide novel information on the mechanisms of neurodegeneration. A similar approach has recently proved useful in defining the role of gut-derived NC stem cells in Hirschprung’s disease (Iwashita et al. 2003).

(iii) Involvement of SKP-like cells in tumours of neural crest-derived tissues

One particularly exciting idea emerges from recent work documenting putative ‘cancer stem cells’ in paediatric neural tumours (Singh et al. 2003, 2004a, b). Such studies raise the possibility that transformed SKP-like ‘neural crest cancer stem cells’ may be important for the genesis of peripheral tumours of the NC. If this were the case, then cultures of transformed SKP-like cells isolated from biopsies could be expanded in vitro for drug screening and optimization of anti-cancer treatments. Conveniently, in at least some instances, tumorigenic and non-tumorigenic precursors could even be isolated from the same individuals, providing an ideal control tissue. In this regard, one candidate disorder is neuroblastoma (NB), which is the most common children’s tumour and the most common extracranial paediatric solid tumour. NB affects migrating early NC precursors of the sympathoadrenal lineage and results in tumours whose primary sites are the adrenal glands and paraspinal sympathetic ganglia, with metastases to bone, lymph nodes, liver and even skin (Kushner 2004; Henry et al. 2005). A second candidate is neurofibromatosis type 1 (NF1; Lakkis & Tennekoon 2000; De Schepper et al. 2005). NF1 is a highly prevalent (1 : 3500) autosomal dominant disorder that results from mutations in the gene encoding neurofibromin. Clinical symptoms of NF1 are primarily observed in NC-derived cells and tissues, and include nerve sheath tumours, melanocytic hamartomas, Café au Lait spots, freckling in sun-protected areas and bone lesions. The presence of Schwann cell, fibroblastic and melanocytic tumour types in NF1 is particularly intriguing in light of the phenotypic instability between these NC-derived cells (Rizvi et al. 2002; Dupin et al. 2003; Real et al. 2005). A third class of disorders that may contain transformed SKP-like cells are tumours of the melanocyte NC lineage. The common dermal dendritic melanocytic proliferations contain embryonic-like spindled and dendritic melanocytes, and the classifications include dermal melanocytic hamartomas, several variants of benign blue naevi, malignant melanocytic tumours (malignant blue naevi) as well as borderline melanocytic tumours that appear to be of mixed neuroectodermal origin (Zembowicz & Milhm 2004).
5. IMPLICATIONS AND UNRESOLVED QUESTIONS

(a) Relationship between SKPs and other skin stem cells

Our findings that SKPs are a multipotent NC-related precursor that is laid down in the dermis during embryogenesis and persists into adulthood in a follicle dermal papilla niche raise a number of intriguing questions concerning possible physiological roles for SKPs.

One potential role for SKPs is in the regulation of the hair and whisker follicle growth cycle, given their niche in the follicle dermal papillae. The dermal cells that reside in these papillae are essential for the hair follicle growth cycle and have therefore been studied extensively. Interestingly, follicle dermal papillae are known to contain a resident precursor cell population: cultured papillary cells can differentiate into adipocytes and osteoblasts (Jahoda et al., 2003), and can even contribute to a small degree to the haematopoietic system in reconstitution experiments (Lako et al., 2002). In vivo, the cells within the follicle dermal papillae undergo marked changes during the hair cycle, increasing in number during anagen stages of hair follicle growth and decreasing during catagen stages of follicle decline. These changes may be partially due to the exchange of cells between the papilla and the nearby follicular connective tissue sheath (Tobin 2003a,b). The papilla cells are essential for hair follicle formation initially during embryogenesis and then on an ongoing basis in mature animals. During the normal hair cycle, potent papilla-derived signals such as Noggin and perhaps Wnts instruct the downward migration of epidermal stem cells residing within the hair follicle bulge, which then differentiate into new hair follicle cells and consequently generate a new hair shaft (Lavker et al., 2003). As a reflection of this essential regulatory role, transplanted dermal papillae are capable of inducing de novo hair follicle formation within the interfollicular epidermis (Reynolds & Jahoda 1992) and play a role in the conversion of adult corneal epithelium to follicular epithelium in response to embryonic dermis (Pearson et al., 2005). Our recent findings, therefore, raise a number of key questions in this regard. First, what proportion of the cells within the follicle dermal papillae are SKPs? Second, are SKPs the same cells as the previously characterized precursor cell population resident in this niche? Finally, are the precursor cells within the follicle papillae the same cells that regulate the hair follicle growth cycle?

SKPs may also provide a resident adult stem cell source for NC-derived cell types in the skin. In the face, the entire dermis is comprised of NC-derived progeny (Le Lievre & Le Douarin 1975; Nakamura 1982), while even in the thoracic regions, the dermis contains many different NC-derived cell types, including Schwann cells, Merkel cells and melanocytes. Intriguingly, Merkel cells are now known to be NC derived (Szeder et al., 2003), and previous work has demonstrated that the dermis contains a precursor cell capable of giving rise to Merkel cells in vivo (Nurse et al., 1984). Could SKPs be this resident dermal NC precursor cell? Given the multipotent nature of SKPs, they might also contribute to the mesodermal components of the dermis, including dermal fibroblasts, either during normal physiological turnover or during wound healing. In this regard, cells of the follicle dermal papillae and sheath have previously been proposed to participate in wound healing in the skin (Jahoda & Reynolds 2001).

In addition to questions regarding their potential physiological role, the elucidation of a novel precursor cell in skin also leads to questions about their interactions with other resident precursor cell populations. For example, MSCs are widespread throughout a variety of tissues (Young et al., 1995), including the dermal layer of the skin, where they are considered the source of the dermal connective tissue. Since MSCs themselves are embryonically derived from either the NC (cranially) or the mesoderm (sub-cranially), further work will be required to clarify the relationship between MSCs and SKPs. One possibility is that SKPs can differentiate in the dermis into a more mesodermally restricted precursor cell with the characteristics of MSCs. A second possibility is that these two populations of cells are independently generated within the dermis, with the MSCs contributing to mesodermal cell types, and SKPs representing a more multipotent precursor that also generates other NC-derived progeny found in the dermis.

Similar issues arise regarding SKPs and other known NC cells resident in the hair follicle. A number of NC-derived cell types are present within the hair follicle bulge and sub-bulge regions, including melanocyte stem cells (Nishimura et al., 2002), recently described epidermal NC stem cells (eNCSCs; Sieber-Blum et al., 2004), and non-innervated Merkel-like cells of unknown function (Narisawa et al., 1994). Melanocyte stem cells are clearly labelled in the bulge/sub-bulge area in dct–lacZ transgenic mice, and these generate transit-amplifying cells that differentiate into mature melanocytes in the hair follicle matrix immediately above the dermal papilla (Nishimura et al., 2002). Little is known regarding the functions of eNCSCs and non-innervated Merkel-like cells, though it has been hypothesized that they may serve a key regulatory function within this critical epidermal stem cell niche (Christiano 2004). Although SKPs and bulge NC-derived cells are present in different layers of the skin, it is possible that they are developmentally related and/or functionally interact, particularly given the regular emigration of cells from the bulge into the lower follicle and from the dermal papillae into the overlying connective tissue sheath (Taylor et al., 2000; Lavker et al., 2003; Tobin 2003a,b; Blanpain et al., 2004; Tumbar et al., 2004). It is even possible that under some conditions SKPs might give rise to more restricted NC precursors within the follicle such as melanocyte stem cells and/or might exchange with the eNCSCs in the bulge region. It also remains to be established whether any of these populations of NC precursors are related to recently described follicular cells that express a nestin–GFP transgene and that are reported to have the ability to differentiate into blood vessels, neurons and myelinating Schwann cells (Li et al., 2003; Amoh et al., 2004, 2005a–c).
(b) Potential origins of SKPs

Our recent data indicate that the first cells with SKP-forming ability arrive in murine back skin at E15, and that their numbers are high during late embryogenesis, decline somewhat into the early post-natal period, and then again in the adult (Fernandes et al. 2004). Given their probable NC origin, the most parsimonious explanation for this time-course is that the relevant NC precursors migrate into skin at E14/E15. In this regard, NC cells could arrive in the skin via a number of migratory routes. In mice, a first wave of trunk NC migration occurs at approximately E9–9.5, when crest cells migrate ventromediately from the neural tube (Serbedzija et al. 1990, 1994; Wilson et al. 2004). These cells generate diverse peripheral nervous system derivatives, including the peripheral nerves that eventually innervate targets that include the skin. Interestingly, multipotent NC stem cells (NCSCs) are present in the rodent peripheral sciatic nerve at E15 (Morrison et al. 1999), the time-point when (i) mouse back skin is first innervated (Peters et al. 2002), (ii) SKPs are first detected in mouse back skin (Fernandes et al. 2004), and (iii) the follicle dermal papillae first form from a population of cells expressing TrkC and p75NTR (Botchkarev et al. 1998, 1999), both of which are expressed on NC precursors (Stemple & Anderson 1992; Luo et al. 2003). Within the peripheral nerve, these NCSCs then apparently disappear by birth (Morrison et al. 1999), something that has been attributed to their terminal differentiation within the nerve itself. However, we propose that a subpopulation of these nerve NCSCs migrate into the skin, participate in the formation of the follicle dermal papillae, and then are maintained as SKPs into adulthood. Indeed, some evidence for nerve-derived NC cells populating the skin has been described in quail–chick chimera experiments (Halata et al. 1990). One prediction of this model is that other peripheral tissues might also contain SKP-like cells that are laid down during embryogenesis when they are first innervated.

Two other NC pathways could contribute NC precursors to skin. First, a second wave of trunk NC migration begins at approximately E10.5 (Serbedzija et al. 1990, 1994; Wilson et al. 2004), when crest cells delaminating from the neural tube shift into a dorsolateral migration pathway, taking them through the developing dermis, from where they ultimately migrate directly into the epidermis of the skin. Cells migrating along this pathway are predominantly of the melanocyte lineage (Wakamatsu et al. 1998). This migration reaches the dermis well before E15, and thus is unlikely to represent the predominant route for SKPs. Another potential source of NC-derived cells in skin is NC boundary cap cells. Boundary cap cells are NC cells that cluster at the sites of spinal and cranial nerve root entry/exit points into the CNS, i.e. the dorsal root entry zone and the ventral motor exit points. Embryonically, boundary cap cells help to regulate the passage of cells and axonal projections between the spinal roots and the CNS (Golding & Cohen 1997; Vermeren et al. 2003), but following the end of NC cell migration from the neural tube, they subsequently migrate distally down peripheral nerves, generating Schwann cells within the spinal roots and nociceptive neurons and satellite cells within the sensory ganglia (Maro et al. 2004; Hjerling-Leffler et al. 2005). Owing to the lack of specific boundary cap markers, it remains unclear how far distally these cells migrate, and whether they enter peripheral target tissues. However, it is possible that boundary cap cells correspond to previously identified cell populations that migrate along peripheral nerves to colonize sensory ganglia and skin (Sharma et al. 1995) and various peripheral target tissues (ventrally emigrating neural tube cells; Ali et al. 1999, 2003a,b; Sohal 1999a,b, 2002) such as trigeminal ganglia, facial cartilage, the gut, heart and vestibular system.

While we have focused here upon potential NC sources for SKPs, we have only formally shown that cranial SKPs are NC derived. Is it possible that non-cranial (thoracic) SKPs express NC-like characteristics without actually being embryologically derived from the NC? In this respect, it is worth noting the following points. First, there are a number of cell types and tissues that are produced developmentally by both the NC and the mesoderm, depending upon their location. For example, bone, cartilage and the entire dermis are produced by NC-derived mesenchymal cells in the craniofacial area (Le Lièvre & Le Douarin 1975) and by mesoderm-derived mesenchymal cells in most of the rest of the body. Consistent with the notion that cells of different embryonic lineages can express similar genetic programmes, a recent study has shown that whisker follicle dermal papilla cells, which are NC derived (Fernandes et al. 2004), behave virtually indistinguishably from bone marrow-derived MSCs in their ability to expand and differentiate into bone, cartilage, fat and muscle (Hoogduijn et al. 2006). Thus, even though back skin dermal papilla express considerable NC-like properties, as further illustrated in a recent gene profiling study (Rendl et al. 2005), it is possible that they are derived from the mesodermal lineage.

Second, consider the examples of the vertebrate neurogenic placodes, particularly the olfactory, trigeminal, otic and epibranchial placodes (Graham & Begbie 2000). Developmentally, the neurogenic placodes are identified as focal regions of thickened cranial ectoderm located outside the neural plate (which is the source of the CNS and NC). These placodes give rise to a large percentage of cranial sensory neurons (including olfactory sensory neurons, trigeminal neurons and vestibular neurons), as well as a number of more specialized cell types (such as glial cells, olfactory epithelium, neuroendocrine cells and sensory hair cells). Thus, during normal development, neurons and glial cells can be generated from areas besides the CNS and NC. Can neurogenic placodes give rise to SKP-like cells? We have previously asked this question focusing upon the olfactory placode-derived olfactory epithelium. Using SKP culture conditions, we have isolated a population of cells from the P14 olfactory epithelium that (i) grows and can be expanded as spheres in response to FGF2 and EGF, (ii) expresses a nestin and fibronectin, and (iii) differentiates into neurons, glia and smooth muscle cells (J.G. Toma,
A. Gloster, and F.D. Miller 1994, unpublished data; figure 3). Similar results have been obtained by others with the human olfactory epithelium (Zhang et al. 2004; Murrell et al. 2005). Thus, placodally derived structures contain a SKP-like cell, raising the possibility that SKPs themselves could potentially derive from an embryonic source other than the NC.

6. CONCLUSIONS
Regardless of embryonic origin, the finding that SKP-like cells reside in the adult olfactory epithelium, together with the possibility that SKPs migrate into peripheral tissues via embryonic peripheral nerves, raises the possibility that SKP-like cells may be present in many peripheral tissues. Further support for this idea derives from a recent report where SKP culture conditions were used to identify a multipotent cell from human dental pulp, a NC-derived tissue (Miura et al. 2003). Whether the SKP-like cells that have been isolated from these different tissues are similar in terms of their potency and/or origin is still an open question, but these findings do raise the provocative possibility that many adult tissues contain multipotent NC-related precursors that were laid down in those tissues during embryogenesis, and that have persisted into the adult.

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REFERENCES


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Hoogduijn, M. J., Gorjup, E. & Genever, P. G. 2006 Comparative characterization of hair follicle dermal stem cells and bone marrow mesenchymal stem cells. Stem Cells Dev. 15, 49–60. (doi:10.1089/scd.2006.15.49)


Jiang, Y. et al. 2002b Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418, 41–49. (doi:10.1038/nature00870)


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