MELANOCYTES/MELANOGENESIS

Key Discoveries in Melanocyte Development

Akinori Kawakami[1] and David E. Fisher[1]

[1]Department of Dermatology, Cutaneous Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

Correspondence: David E. Fisher, E-mail: dfisher3@partners.org

doi:10.1038/skinbio.2011.2

Melanocytes are melanin pigment-producing cells. Mammalian melanocytes are categorized as ''cutaneous'' (follicular and epidermal) and ''extracutaneous'' (e.g., choroidal, cochlear). Epidermal melanocytes contribute to photoprotection and thermoregulation by packaging melanin pigment into melanosomes and delivering them to neighboring keratinocytes. Melanocytes are derived from the neural crest that is a migratory multipotent population that gives rise to multiple cell lineages, including neurons, glial cells, medullary secretory cells, smooth muscle cells, and bone and cartilage cells. Coat color mutants in different species have been useful for identifying genes involved in melanocyte development.

Embryonic transplantation experiments played a significant role in early efforts to investigate melanocyte development. Rawles (1947) conducted a series of elegant transplantation experiments, which revealed that melanocytes originate from the neural crest. The investigator transplanted various axial levels of the embryonic central nervous system, adjacent tissues of the somite and lateral plate, and limb-bud regions, separately and in combination, from various developmental stages of black mouse embryos, to the coelom of White Leghorn chick embryos. Only tissues containing the neural crest or cells migrating from the neural crest were found to produce melanophores. In addition, Rawles found that the portions of embryo that produce pigment cells vary at different developmental stages of donor embryos.

The quail nucleus exhibits condensed heterochromatin, which could serve as a marker to distinguish quail cells from chick cells. Teillet and Le Douarin (1970) studied melanoblast migration from the neural crest using a quail-chick xenograft transplantation model. The investigators transplanted different axial levels of quail neural tube and neural crest to White Leghorn chick embryos. They found that (1) at embryonic days E4 and E5, the transplanted quail cells localize mainly in the mesenchyme; (2) at E6 when the dermis and the epidermis are formed, quail cells (melanoblasts) begin to migrate into the epidermis; (3) at E9 quail cells (melanoblasts) increase their cytoplasmic volume and start producing melanin pigments; (4) at E10 and E11 quail cells (melanoblasts) localize in the basal layer of epidermis and become dendritic.

Cell type-specific markers are useful tools to study the development of certain cell types. However, it is challenging to identify these markers. Steel *et al*. (1992) found Tyrp-2/Dct to be a specific marker of melanoblasts, the precursors of melanocytes. Using *in situ* hybridization, the investigators found that Tyrp-2 expression was detectable in melanoblasts as early as 10 days post coitum.

The finding of a marker for early melanoblasts enabled scientists to look for the mechanisms of coat color mutations. *Steel* and *W* mutants exhibited a white spotting color pattern. *W* and *Steel* encode, respectively, a receptor tyrosine kinase, Kit, and its ligand, which is known by several different names: steel factor, stem cell factor, mast cell growth factor, and Kit ligand. The mutation Steel-dickie (Sl^d) is a deletion of its transmembrane and cytoplasmic domains so that only a secreted form of stem cell factor is produced. Steel *et al*. (1992) found that the number of melanoblasts in (Sl^d/Sl^d) mutants began to decrease at around 11 days post coitum. They also found that the melanoblasts caudal of the optic vesicle failed to migrate toward the vesicle. These results suggested that the cell surface form of stem cell factor is important for both the survival and migration of melanoblasts. Wehrle-Haller and Weston (1995) performed *in situ* hybridization with Kit, Tyrp-2, and stem cell factor probes in *Sl* (null) and Sl^d mutants to examine the early dispersal and fate of melanoblasts in order to elucidate the function of stem cell factor in more detail. They concluded that soluble stem cell factor is sufficient for responsive melanoblast precursors to initiate their dispersal onto the lateral migration pathway, and that cell-bound stem cell factor was necessary for the survival of melanoblasts in the newly formed dermal mesenchyme.

Dorsky *et al*. (1998) found that cranial neural crest cells destined to encode pigment cells were located adjacent to the Wnt-1 and Wnt-3a expression domain, whereas neurons were far from the Wnt-expressing domain in zebrafish. Most lateral cells, which become neurons when forcibly overexpressing an activated form of β-catenin, adopted a pigment-cell fate. Conversely, when the investigators overexpressed a mutant form of Tcf-3 or a dominant-negative Wnt-1 to inhibit Wnt signaling in medial neural crest cells, the number of pigment cells decreased dramatically. Thus, Dorsky *et al*. provided key evidence that Wnt signaling plays an essential early role in pigment cell formation.

Mutations in both *Ednrb* and *Edn3*, *piebald-lethal* (*Ednrb*$^{s-l}$), and *lethal spotting* (*Edn3*^{ls}) exhibit severe melanocyte defects. To investigate the function of EDNRB signaling during melanocyte development, Shin *et al.* (1999) expressed or repressed *Ednrb* expression using a tetracycline-inducible system. Their results indicated several important points: (1) *Ednrb* is required for melanocyte development between E10 and E12.5; (2) *Ednrb* may not be required for premigratory melanocytes; (3) *Ednrb* may be required for the initiation of melanoblast migration and/or for their survival; (4) Ednrb is not required for postmigratory epidermal proliferation, survival, and differentiation of melanoblasts.

MITF is a master transcriptional regulator of the melanocyte lineage. MITF mutations cause Waardenburg syndrome type 2a, an autosomal dominant (heterozygous) condition that is characterized by deafness and partial depigmentation due to localized deficiencies of melanocytes. Mutations at the mouse *Mitf* locus show melanocyte defects in the skin, eyes, and inner ears. MITF's transcriptional target genes include lineage survival factors (e.g., apoptosis regulators) as well as pigmentation-related genes (several of which are implicated in albinism). Yasumoto *et al.* (1994) found that MITF specifically activates the transcription of tyrosinase through a consensus DNA motif E-box (CA[C/T]TG) in the melanocyte lineage. Hemesath *et al.* (1994) found that Mitf bound the same E-box as either a homodimer or a heterodimer with TFEB, TFEC, or TFE3, and proposed recognition of these factors as a distinct ''MiT'' transcription factor family. MITF, TFEB, and TFE3 have also been implicated as amplified or translocated oncogenes in a variety of human malignancies, including melanoma, soft tissue sarcomas, and renal carcinomas.

More recent studies have sought to identify and functionally characterize melanocyte stem cell populations. Nishimura *et al.* (2002) identified melanocyte stem cells and their niche using Dct-lacZ transgenic mice, which carry the *lacZ* reporter under the control of the *Dct* promoter, and ACK2, a blocking c-Kit targeted monoclonal antibody. The investigators found that melanocyte stem cells reside in the bulge region of the hair follicle (lower permanent portion of the follicle) and fulfill the criteria for stem cells: immature, slow cycling, and self-maintaining, with an ability to regenerate progeny on activation at early anagen. Subsequently, Nishimura *et al.* (2005) observed that age-related hair graying is associated with defective self-maintenance of melanocyte stem cells and physiological aging/depletion of melanocyte stem cells caused by ectopic pigmentation or differentiation within the niche.

Melanoblasts have, for many years, been thought to arise from the dorsal neural tube and migrate dorsolaterally between the dermamyotome and the ectodermatome and ventrally through the developing dermis to their final destination, the basal layer of the epidermis, and the hair follicles. However, melanoblasts may localize to the limbs without a dermal migration. Adameyko *et al.* (2009) conducted an elegant combination of experiments to address this question and reassess the origin of melanocytes; they provided compelling new evidence that Schwann cell precursors may serve as the cells of origin for a major fraction of skin melanocytes (Figure 1).

Our knowledge of melanocyte development has been expanding rapidly, but many important questions remain unanswered. How do signals and transcription factors cooperate during melanocyte development? How do the cells surrounding melanoblasts contribute to melanocyte development? How does the epigenetic status change at different stages of melanocyte development? Are melanocytes of different locations functionally the same, and may they be interchanged for clinical benefit? Uncharacterized pigmentation mutants and new technologies, such as chromatin immunoprecipitation sequencing and the melanocyte lineage-specific targeted knockout mice (e.g., Tyr::CRE, Dct::CRE), are permitting ever-expanding answers to these questions.

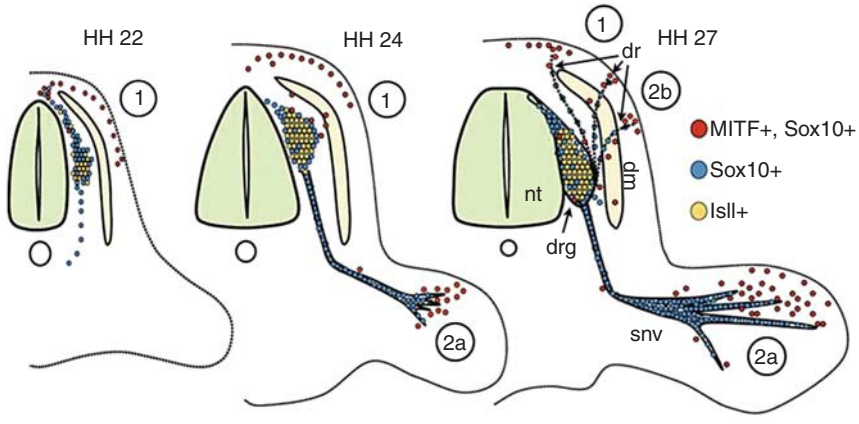

Figure 1. Migratory pathways of chick trunk neural crest cells, adapted from *Cell* 2009; 139:366–379. Region 1: Melanoblasts (Mitf/Sox10 positive cells) migrating within the dorsolateral pathway (the ''classic'' melanoblast migration route). 2a: Melanoblasts located near ventral spinal nerves. 2b: Melanoblasts along nerves of dorsal rami. nt, neural tube; drg, dorsal root ganglion; dm, dermamyotome; dr, dorsal ramus; snv, ventral branch of the spinal nerve; HH, Hamburger Hamilton stage.

CONFLICT OF INTEREST

The authors state no conflict of interest.

TO CITE THIS ARTICLE

Kawakami A, Fisher DE (2011) Key discoveries in melanocyte development. *J Invest Dermatol* 131: E2–E4

REFERENCES

Adameyko I, Lallemend F, Aquino JB *et al.* (2009) Schwann cell precursors from nerve innervation are a cellular origin of melanocytes in skin. *Cell* 139:366–79

Dorsky RI, Moon RT, Raible DW (1998) Control of neural crest cell fate by the Wnt signalling pathway. *Nature* 396:370–3

Hemesath TJ, Steingrímsson E, McGill G *et al.* (1994) Microphthalmia, a critical factor in melanocyte development, defines a discrete transcription factor family. *Genes Dev* 8:2770–80

Nishimura EK, Granter S, Fisher DE (2005) Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. *Science* 307:720–4

Nishimura EK, Jordan SA, Oshima H *et al.* (2002) Dominant role of the niche in melanocyte stem-cell fate determination. *Nature* 416:854–60

Rawles ME (1947) Origin of pigment cells from neural crest in the mouse embryo. *Physiol Zool* 20:248–70

Shin MK, Levorse JM, Ingram RS *et al.* (1999) The temporal requirement for endothelin receptor-B signalling during neural crest development. *Nature* 402:496–501

Steel KP, Davidson DR, Jackson IJ (1992) TRP-2/DT, a new early melanoblast marker, shows that steel growth factor (c-kit ligand) is a survival factor. *Development* 115: 1111–9

Teillet MA, Le Douarin N (1970) The migration of pigmentary cells studies by the method of heterospecific grafts of neural tube in bird embryo. *C R Acad Sci Hebd Seances Acad Sci D* 270:3095–8

Wehrle-Haller B, Weston JA (1995) Soluble and cell-bound forms of steel factor activity play distinct roles in melanocyte precursor dispersal and survival on the lateral neural crest migration pathway. *Development* 121: 731–42

Yasumoto K, Yokoyama K, Shibata K *et al.* (1994) Microphthalmia-associated transcription factor as a regulator for melanocyte-specific transcription of the human tyrosinase gene. *Mol Cell Biol* 14:8058–70