

Lessons from melanocyte development for understanding the biological events in naevus and melanoma formation

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Recent advances in mouse genetics have identified molecular changes that are critical for melanocyte maturation and differentiation. This review briefly summarizes the current knowledge of distinct steps in melanocyte development, and identifies for each step the most important molecules such as the growth factors stem cell factor and endothelin-3, with their respective receptors. Classical cadherins, i.e. E-cadherin, N-cadherin and P-cadherin, determine melanocyte positioning in the skin. During naevus and melanoma development, the two growth factor signalling pathways are downregulated, whereas cadherin expression shifts concomitantly with re-positioning of the naevus and melanoma cells in the skin. Loss of E-cadherin and gain of N-cadherin by melanoma cells has profound consequences for the regulatory cross-talk between various types of cells in the skin. Naevus and melanoma cells that do not express E-cadherin are resistant to control by keratinocytes and establish close communications with fibroblasts and endothelial cells. However, forced expression of E-cadherin in melanoma cells can reverse the malignant phenotype by re-establishing the control of keratinocytes over the melanoma cells. Even highly aggressive metastatic melanoma cells can be signalled to turn off the expression of genes associated with tumour invasion and metastasis, suggesting that this strategy could be utilized in the therapy of melanoma. © 2000 Lippincott Williams & Wilkins

Key words: melanocyte, melanoma, naevus, neural crest migration

Introduction

The melanocyte in human skin is normally embedded in the basal layer of keratinocytes, anchored to the basement membrane of the epidermis. Each melanocyte extends its dendrites into the upper layers of the epidermis and establishes contacts with keratinocytes, forming the 'epidermal melanin unit' that comprises 20 to 35 cells. On ultraviolet (UV) light stimulation, pigment in melanosomes is trans-

ferred from the melanocyte dendrites to the keratinocytes by an undefined mechanism. During expansion of the total skin surface in childhood there is a continuous need for melanocyte proliferation to maintain a stable ratio with the keratinocytes in the basal layer. In adults, melanocytes are generally resting, but they are still capable of proliferating, albeit at a slower rate. They can be grown *in vitro* for several passages, but they senesce more rapidly than melanocytes from young donors. Melanocyte precursors, melanoblasts, have been identified in animals; their existence in humans is unclear, but most likely.

This brief review will highlight the events during melanocyte development, particularly during the dorsolateral migration of neural crest progenitor cells from the neural tube. Recent advances in mouse genetics have identified genes whose functions are essential for melanocyte precursor migration and differentiation. Not surprisingly, abnormal growth and invasion during naevus and melanoma development retraces many of the critical developmental steps. We will point to the similarities between melanocyte development and malignancy, with emphasis on the shifts in expression of cell-cell adhesion receptors during melanocyte re-positioning in tissues.

Melanocytes in different mammalian species are found in different locations in the skin. In mouse skin, melanocytes are located in the hair follicles and dermis and are absent from the epidermis. In human skin, on the other hand, melanocytes are positioned in the epidermis and hair follicles. This positioning of human melanocytes leads to intimate adhesions and intercellular communications with keratinocytes, which are lost after melanocyte transformation. We will discuss some of the molecules and events that appear to be critical for melanocyte development, migration and proliferation, and for naevus and melanoma formation.

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Melanocyte development in the mouse

Melanocytes are derived from progenitor neural crest cells. Their migration and differentiation during development has been studied in several species, but in most detail in the mouse.¹⁻³ Besides the published literature, most recent studies were summarized at the XVIIth International Pigment Cell Conference, October 30–November 3, 1999, in Nagoya, Japan.⁴ Between mouse embryonic days 8 and 14 there are at least four distinct developmental steps leading to the appearance of fully differentiated melanocytes (Table 1). Neural crest cells migrate from the neural tube to arrive at the dorsolateral surface by day 8.5. These cells mature into a precursor cell type that has the potential to differentiate into either melanoblasts or glial cells. Melanoblast precursors can differentiate into glial precursors and, potentially, glioblasts can differentiate into melanoblasts. This common origin for melanocytes and glial cells explains why glioma and melanoma cells share many tumour-associated markers and have many similar biological properties. Melanoblast precursors mature into melanoblasts; these are committed cells staining for the melanocyte-specific marker tyrosinase-related protein (TRP)-2. They are still non-pigmented and thus are negative for TRP-1 and tyrosinase. Under the influence of melanocyte-stimulating hormone (MSH) melanoblasts then differentiate into pigmented melanocytes, which express the classical differentiation markers, TRP-1 and tyrosinase.

Cadherins appear to determine the location of the melanocytes in the skin. During migration, melanocyte precursors do not express E- or P-cadherin; it is unknown whether they express N-cadherin. As the melanocytes migrate into the epidermis at approxi-

mately day 11.5, E-cadherin expression increases almost 200-fold.⁵ E-cadherin expression then decreases and the melanocytes leave the epidermis to migrate to the hair follicles and dermis. In the hair follicle, melanocytes express P-cadherin strongly but little or no E-cadherin. In the dermis, melanocytes lack both P- and E-cadherin, but express N-cadherin.

We will not discuss in detail the roles of the transcription factors microphthalmia-associated transcription factor (MITF), paired-box gene-3 (PAX-3) and SRY box-containing gene 10 (Sox10). If any of these are absent, as established through natural mutations or gene knock-out studies, melanoblasts are either absent (Sox10) or greatly reduced (MITF and PAX-3). In the following, we will focus on three molecular pathways, stem cell factor (SCF)/*c-kit*, ET-3/ETRB, and E-cadherin/ β -catenin, which are all essential for melanocyte development, survival and growth. These pathways share similarities in that they are inoperative in melanoma; however, only the E-cadherin/ β -catenin pathway is sufficiently understood for its significance in melanoma to be assessed.

The SCF/*c-kit* signalling pathway

The growth factor SCF is also known as steel factor, mast cell growth factor or kit ligand.^{6,7} It is the gene product of the murine steel locus (Sl) on chromosome 10. Loss of SCF in mice leads to the absence of melanoblasts. *Sld* (*dickie*) germline mutants lack the transmembrane and cytoplasmic domains of wild-type SCF and are viable but anaemic, non-pigmented (black-eyed white), deficient in mast cells, and sterile.⁸⁻¹³ SCF is highly mitogenic for human melanoblasts and melanocytes in culture and for melanocytes *in vivo*.^{14,15}

Table 1. Expression of various factors during melanocyte development in the mouse

Factor	Day of mouse development			
	8 (neural progenitor cell)	10 (precursor melanoblast)	12 (glioblast/melanoblast)	14 (melanocyte)
<i>c-kit</i>	–	+	+	+
ETRB	+?	+	+	+
TRP-2	–	–	+	+
TRP-1	–	–	–	+
Tyrosinase	–	–	–	+
E-cadherin	–	–	Epidermis + ^a	}}-
P-cadherin	–	–	–	Hair follicle + ^b
N-cadherin	?	?	–	Dermis + ^c

^aExpression of E-cadherin restricted to melanoblast in epidermis.

^{b,c}Expression of P-cadherin and N-cadherin restricted to hair follicle or dermis, respectively.

SCF is composed of extracellular, transmembrane and cytoplasmic domains and exists in membrane-bound and soluble forms (Figure 1A). The soluble form is released by cleavage at a protease-sensitive site. SCF can be produced in the skin by keratinocytes, fibroblasts and endothelial cells. In the mouse, unlike in humans, epidermal keratinocytes do not express SCF postnatally, although it is produced by other cells in the dermis and hair follicles. The presence of melanocytes in the dermis and hair follicles suggests that the continuous presence of SCF is essential for melanocyte survival and maintenance. Mice that express membrane-bound and soluble SCF in keratinocytes show dermal mast cell infiltrates and epidermal hyperpigmentation.^{8,9,11,12}

When, as in human skin, murine epidermal keratinocytes express only the membrane-bound form of SCF, melanocytes are maintained in the epidermis, but mastocytosis does not develop.⁸ Thus, the membrane-bound form of SCF seems necessary for a normal phenotype of melanocytes in human skin.

The receptor for SCF is *c-kit*, a receptor tyrosine kinase encoded by the *c-kit* proto-oncogene (Figure 1B). The extracellular domain of the 145 kDa protein comprises five immunoglobulin-like loops, of which the second loop contains the ligand-binding domain and the fourth the dimerization domain. The cytoplasmic tail contains two kinase domains. The structure of the 170 kDa platelet-derived growth factor receptor- α (PDGFR α) is nearly identical to *c-kit*, except that signalling through the kinase domains differs substantially. Both genes are juxtaposed on chromosome 4q 1.1–1.2. *c-kit* is the gene product of the dominant white spotting locus (the W locus). Mutations of the W locus lead to pigmentation defects, sterility and anaemia. Complete loss of *c-kit* results in loss of melanocytes, primordial germ cells and haematopoietic stem cells.

Expression of *c-kit* in melanoma cell lines and tumours is downregulated with progression.¹⁶⁻²¹ Cultured melanoma cells generally do not respond to SCF. Treatment of *c-kit*-expressing melanoma cells with SCF leads to apoptosis,^{22,23} suggesting that there are profound aberrations in signalling during progression. The downregulation of *c-kit* correlates with a decrease in the transcription factor AP-2,²⁴ which binds to the *c-kit* promoter. Conversely, transduction of AP-2 into melanoma cells induces *c-kit* expression. The mechanisms of the downregulation of *c-kit* in melanoma remain to be clarified. There are no apparent gene defects, but rather a transcriptional downregulation of expression that appears to be critical for the emergence of transformed cells.

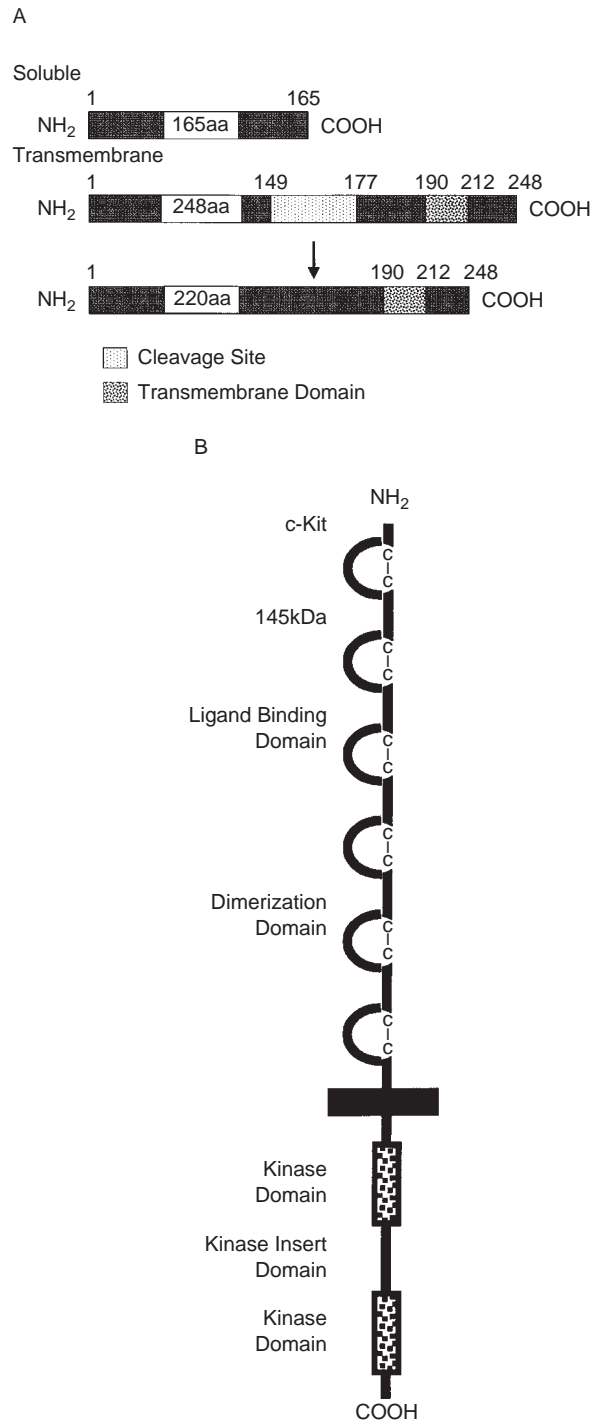


Figure 1. A Schematic representation of SCF isoforms in humans. Two isoforms of SCF, soluble (SCF165) and membrane bound (SCF220), are depicted. Alternate splicing can generate soluble or transmembrane forms; both retain their biological properties. Absence of exon 6 (amino acid 149 to 177) results in the membrane-bound form, while proteolytic cleavage at amino acid 165 from SCF248 results in the soluble form. **B** Schematic representation of *c-kit* receptor. *c-kit* is a type I transmembrane protein 273 amino acids in length. It contains five immunoglobulin-like domains in the extracellular region, which phosphorylates tyrosine residues of the target proteins after activation by the ligand binding.

Endothelins 1 and 3 and their receptors

In humans there are three members of the endothelin (ET) peptide family (ET-1, ET-2 and ET-3) and two ET receptors (ETRA and ETRB). ETRA displays high and equal affinity for ET-1 and ET-2, and an approximately 70- to 100-fold lower affinity for ET-3 (Figure 2). ETRB has an equally high affinity for all ET-related peptides. ET-3 is the gene product of the lethal spotting (*ls*) locus and ETRB is the gene product of the piebald lethal (*s^l*) locus.^{25,26} Mice with mutations in either loci have pigment defects (white spotting) and a ganglionic megacolon, indicating that neural crest cells of different lineages are affected. ETRB is required for the regulation of precursor melanoblast numbers and is the more important receptor in the initial stages of melanocyte development when compared with *c-kit*.²⁷⁻³⁰ Characterization of the development of melanocytes in knock-out mice has provided insight into the functions of ET-1 and ET-3 and their receptors. ET-1^{-/-} mice die at birth, but have no pigmentation defect.³¹ Thus, ET-1 does not appear to be essential for precursor melanocytes during early development and may be fully compensated by ET-3. ETRA^{-/-} mice have a virtually identical phenotype to ET-1^{-/-} mice.³² ET-1 is expressed in skin during initiation of pigmentation and may be responsible for the pigment observed in ET-3^{-/-} mice.

ET-3^{-/-} mice are predominantly white and have normal tissue levels of ET-1 and ET-2, indicating that the latter do not compensate for the loss of ET-3. However, ET-3^{-/-} mice have more pigment in the head and tail region than ETRB^{-/-} mice indicating that the other endothelins may partially compensate for the loss of ET-3 during differentiation of progenitor melanocyte.^{33,34} ET-3 is involved in the regulation of progenitors in the dermis, but its importance in the later stages is unclear. The role of ET-2 is unknown and there are no ET-2^{-/-} mice available to date.

Melanoma cells express both ETRA and ETRB.³⁵ However, when compared with melanocytes, they express decreased levels of ETRB.³⁶⁻³⁸ ET-1, similar to SCF, can induce apoptosis in melanoma cells,³⁹ which have been found to express frequently a truncated form of ETRA.⁴⁰ The biological function of this splice variant is not known. It appears that the presence of ET receptors on melanoma cells is, similar to *c-kit*, detrimental to melanoma cell growth and survival. However, deregulated expression and function of ET receptors at any particular stage of melanoma progression remains to be precisely defined. A better understanding of this switch may provide further information for a new therapeutic strategy for melanoma.

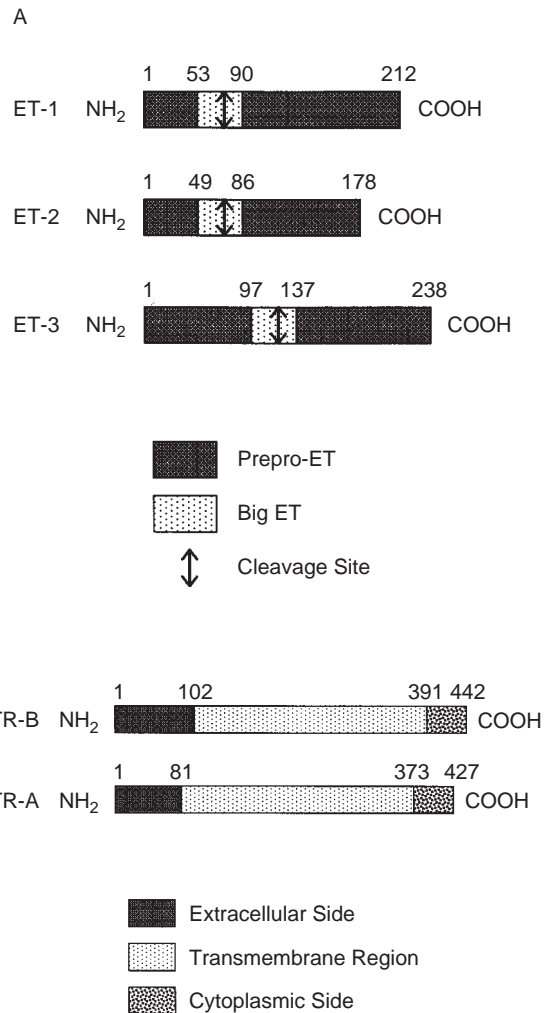


Figure 2. A Schematic protein representation of ETs in humans. The boxes symbolize the prepro-ETs, which are about 200 amino acids in size. The exact size of each protein is shown by the numbers above the boxes. The prepro-ETs are intracellularly processed by cleavage of paired basic sites by furin-type proteases into intermediate (38–41 amino acids) inactive peptides termed big ETs. ET-converting enzymes then process the big ETs, generating bioactive 21 amino acid peptides. ET-1 and ET-2 are formed by cleaving a Trp21–Val22 bond, and ET-3 is generated by cleaving a Trp21–Ile22 bond. The gene for ET-1 is located on chromosome 6, for ET-2 on chromosome 1, and for ET-3 on chromosome 20. **B** Schematic protein representation of ETRA and ETRB in humans. ET receptors are transmembrane G protein-coupled receptors. The boxes symbolize the proteins, which are 442 and 427 amino acids in size, respectively. The transmembrane region consists of seven α helices (TM1-7). The gene for ETRB is located on chromosome 13 and for ETRA on chromosome 4.

Cadherins in development

The cadherins form a family of cell surface glycoproteins that function in promoting homophilic, calcium-dependent cell-cell adhesion, and serve as

transmembrane components of cell-cell adhesion junctions.⁴¹⁻⁴⁴ The classical cadherins, E-, P- and N-cadherins, have similar structures (Figure 3). The extracellular domain contains five immunoglobulin-like repeat units termed EC1 through EC5. The HAV peptide in the EC1 domain mediates homotypic binding. The cytoplasmic domain binds β -catenin, γ -catenin (plakoglobin) and p120. The catenin molecules are important signalling mediators for the cadherins. In melanoma, β -catenin is mutated in approximately 5–25% of cases,^{45,46} leading to constitutive stabilization and activation of the catenin signalling pathway. Even without mutations, β -catenin appears to be constitutively activated in melanoma.

Cadherin molecules and cadherin-containing complexes are involved in cell recognition, motility, tissue integrity, and homeostasis during embryogenesis and morphogenesis, all of which have been studied in the development of several organisms. Various approaches have been explored to assess the functions and significance of cadherins. These include administration of blocking antibodies or peptides,⁴⁷ forced expression of dominant-negative cadherins,⁴⁸ inducible ectopic expression of cadherins in transgenic animals,⁴⁹ and the use of knock-out animals. These studies have established the roles of cadherins in the formation of tissues and organs in the developing embryo. Cadherins are involved in the epithelial-mesenchymal interactions that lead to skin and muscle formation, the regulation of cell survival/apoptosis in tissues undergoing active morphogenesis, and the establishment and organization

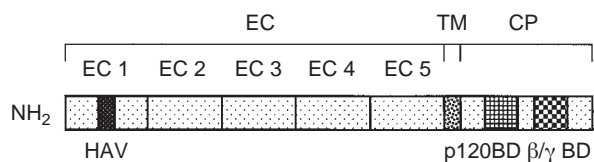


Figure 3. Linear schematic representation of classical cadherin molecules in humans showing the functional domains. Cadherins are single-pass transmembrane proteins with the N-terminus forming the extracellular (EC) domain and the C-terminus forming the cytoplasmic (CP) domain. EC1 through EC5 represent the five extracellular repeat units, which are each about 110 amino acids in length. The HAV region of EC1 is required for calcium binding and homotypic interaction. TM represents the transmembrane domain. The cytoplasmic domain of cadherins contains binding domains (BD) that interact with the Armadillo family of proteins such as β -catenin, γ -catenin (plakoglobin) and p120. The EC domains, which are believed to determine the specificity of cadherin-mediated interactions, are relatively less conserved among different cadherins compared with the CP domains. Specific amino acids are not identified in the figure because they are dependent on individual cadherins, though the general rule holds as described above.

of the central nervous system. In mouse skin morphogenesis and homeostasis, the three classical cadherins, E-, N- and P-cadherin, play important roles. The phenotypes for cadherin knock-outs range from severe, i.e. lethality at a very early stage of development for E-cadherin,^{50,51} less severe, with lethality before day 10 of gestation for N-cadherin,^{52,53} to minor for P-cadherin.⁵⁴ Another class of ligands belonging to the Wnt (wingless) family, which in part signals through β -catenin, has been shown to be important for melanocyte development. Genetic deletion of a specific member of the Wnt family leads to complete loss of melanocytes.⁵⁵

In normal human skin, E-cadherin is expressed on the surfaces of all epidermal cells, including keratinocytes, melanocytes and Langerhans cells, while P-cadherin is expressed only on the surface of basal layer keratinocytes. During human skin development, P-cadherin expression is spatiotemporally controlled and is closely related to the segregation of the basal layers as well as to the arrangement of epidermal cells into eccrine ducts.^{56,57} N-cadherin is expressed in the human skin by fibroblasts and endothelial cells, but not keratinocytes or melanocytes.⁵⁸

Keratinocytes control normal melanocytes but not melanoma cells

Important clues regarding cellular homeostasis in controlling aberrant melanocytic cell growth has come from co-culture experiments. When melanocytes are maintained in culture they express a variety of cell surface molecules that are absent on melanocytes in skin. These molecules are of diverse origin,⁵⁹ we have studied the expression of the vitronectin receptor $\alpha v \beta 3$ and the cell-cell adhesion receptor Mel-CAM in most detail.^{60,61} Normal melanocytes *in vitro* have a phenotype similar to melanoma cells. When the melanocytes are co-cultured with keratinocytes, expression of these melanoma-associated antigens is lost within 3–4 days, suggesting that the keratinocytes control cell surface molecules on the melanocytes. On the other hand, melanoma cells are refractile to the regulatory activity of keratinocytes.

Keratinocytes also control melanocyte proliferation. When keratinocytes and melanocytes are seeded together at a fixed ratio and allowed to proliferate, the original ratio remains constant during proliferation of both cell types, suggesting that the

keratinocytes regulate their equilibrium with the melanocytes. The regulatory activity of keratinocytes occurs through direct cell-cell contact and not through soluble factors.^{59,60} Furthermore, keratinocytes must be maintained in low calcium-containing medium, indicating that only keratinocytes resembling basal layer cells are capable of regulating the phenotype of melanocytes.⁵⁹ We now have evidence that E-cadherin-mediated adhesion between the two cell types is critical for intercellular signalling;⁶¹ however, it remains to be determined whether the signals between the cells are transmitted through E-cadherin only or through another cell-cell adhesion system, i.e. a co-receptor. Intercellular adhesion between melanocytes and keratinocytes leads to the establishment of gap junctions between these two cell types.⁶² When the melanocytes lose E-cadherin expression, which usually occurs during prolonged culture *in vitro* (approximately above passage 6 to 8), the keratinocytes are not capable of controlling them. Keratinocytes are also unable to control the growth and antigen phenotype of melanoma cells or of other normal cells in the skin such as fibroblasts or endothelial cells.

Steps of melanocyte proliferation in normal human epidermis

The biological events of melanocyte proliferation in normal skin are not known. Melanocytes embedded in the epidermis among the basal layer keratinocytes do not proliferate, but maintain their proliferative capacity. For the highly dendritic melanocytes to proliferate we suggest the following distinct steps need to occur (Table 2).

Step 1: Downregulation of E-cadherin

This downregulation leads to decoupling of melanocytes from keratinocytes. Potentially, E-cadherin can

be downregulated through increased production of scatter factor/hepatocyte growth factor (SF/HGF) by fibroblasts.⁶³ Alternatively, UV irradiation can downregulate E-cadherin by upregulation of ET-1 expression in keratinocytes and/or by upregulation of a yet to be identified DNA methyltransferase. E-cadherin on keratinocytes may also be affected instead of E-cadherin expressed by melanocytes.

Step 2: Loss of gap junction

Downregulation of E-cadherin leads to loss of gap junctional communication. The functional consequences of this step are not known.

Step 3: Retraction of dendrites

Melanocytes with multiple dendrites extending far into the epidermis are not expected to proliferate. It is possible that they retract the dendrites by regulating rac-1,⁶⁴ which is a member of the rho family of GTPases. Regulation of these GTPases is poorly understood, but we suggest that physical forces such as increased tissue tension and microenvironmental cues could be initiating factors.

Step 4: Induction of proliferation

Proliferation of melanocytes that are now without dendrites and decoupled from the keratinocytes could be induced by any of the melanocytic mitogens that are produced by fibroblasts (SF, basic fibroblast growth factor [bFGF]) or keratinocytes (SCF, ET-3, bFGF, leukotrienes). We suggest that the cell-bound SCF is the most likely mitogen when it is released from the keratinocyte membrane through enzymatic cleavage.

Table 2. Hypothetical steps in normal human melanocyte proliferation during childhood

Step	Function	Molecule	Induction
1	Cell-cell adhesion	E-cadherin↓	SF from fibroblasts↑
2	Gap junction	Connexin43↓	E-cadherin↑
3	Dendrite formation	rac-1↓	Tension
4	Proliferation	SCF	Enzymatic cleavage
5	Migration along basement membrane	α6β1↓	TGFβ activation
6	Homeostasis	E-cadherin↓	

↑, decrease in expression; ↓, increase in expression; TGFβ, transforming growth factor-β.

Step 5: Migration

After dividing, melanocytes have to separate from each other because they are usually situated individually along the basement membrane with five to eight keratinocytes between them. The integrins that anchor the melanocytes to the basement membrane are not established. We suggest that the laminin receptor $\alpha6\beta1$ is the most important receptor for migration rather than one of the collagen receptors. It can be speculated that the Notch signalling system plays a role in positioning. The transmembrane Notch receptor is relatively ubiquitously expressed and may be activated by one of its ligands, Delta or Jagged, depending on the ratio of melanocytes to keratinocytes.⁶⁵ This repositioning for homeostasis is critical, because failure can lead to escape of melanocytes, continuous proliferation and naevus formation.

Step 6: Homeostasis

E-cadherin becomes functional again to allow the re-establishment of intercellular communication.

Cadherins in naevus and melanoma formation

Whereas all the melanocytes we have tested express E-cadherin *in vitro* and *in vivo*, melanoma cells, with few exceptions, do not (Figure 4). Instead, melanoma cells express N-cadherin.⁶⁴ Naevus cells are generally negative for E-cadherin and positive for N-cadherin (unpublished data), but extensive immu-

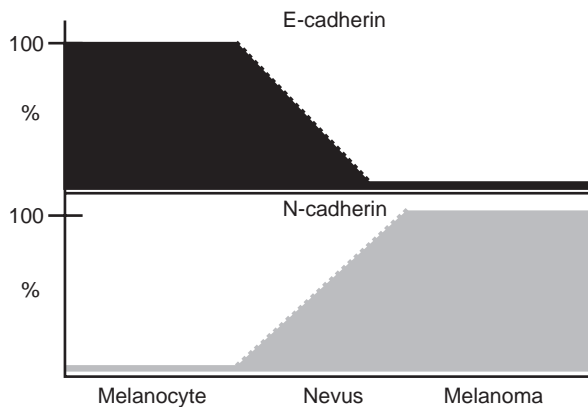


Figure 4. Expression of E-cadherin and N-cadherin by human melanocytic cells at different stages of tumour progression. The hatched line indicates that naevus cell expression for both molecules has not been investigated in detail.

nohistochemical analyses have not been performed. The expression of N-cadherin allows the melanoma cells to form gap junctions with fibroblasts and endothelial cells.⁶¹ It remains to be investigated which biological consequences result from the establishment of gap junctions between naevus or melanoma cells and fibroblasts or endothelial cells.

Forced expression of E-cadherin by melanoma cells through gene transduction followed by co-culture with keratinocytes has profound effects on the phenotype of melanoma cells.⁶¹ E-cadherin-expressing melanoma cells can adhere to keratinocytes. After co-culture with keratinocytes, melanoma cells no longer express the invasion-related adhesion receptors $\alpha v\beta 3$ or Mel-CAM and lose their invasive capacity (Table 3). Keratinocytes can then regulate the growth of melanoma cells in the same manner as melanocytes and gap junctions are established between the melanoma cells and keratinocytes. These results demonstrate that even metastatic cells can develop a phenotype of normal melanocytes when the keratinocytes are allowed to establish intimate adhesive interactions.

Future studies

The dynamics of expression of growth factors, their receptors and of adhesion molecules during melanocyte maturation is beginning to be understood due to extensive studies in the mouse embryo. However, while the initial stages of development may be the same in mice and humans, advanced stages of maturation and differentiation are expected to be different because of architectural and functional differences. Mouse genetic approaches can only provide approximate information on melanocyte biology in humans. The mouse is also a poor model to study naevus formation or early stages of melanoma unless in future studies mouse melanocytes can be genetically positioned exclusively in the epider-

Table 3. Human keratinocytes influence E-cadherin-transduced human melanoma cells in co-culture experiments

Characteristic	Co-culture	
	Before	After
Melanoma-CAM	High	Negative
$\alpha v\beta 3$	High	Negative
Invasiveness in reconstructs	High	Low
Attachment to keratinocytes	Poor	Rapid
Growth regulation	No	Yes
Gap junction	No	Yes

mis and hair follicles. Therefore, an experimental model of human melanoma development using human skin grafted to immunodeficient mice has been developed.⁶⁶⁻⁶⁸ However, this model has limitations stemming from the immunodeficient environment of the host, the lack of cross-reactivity of some murine cytokines to the human receptors, and the low incidence of melanoma development.

Skin reconstruction models in which the melanocytes are maintained in organotypic culture will allow the development of models to study the dynamic changes when a fully differentiated melanocyte proliferates and forms a naevus. Knowledge about the major driving forces for maturation and differentiation are the cornerstone for skin models that resemble transgenic and knock-out models in the mouse. The dynamics of the changes in cadherin expression during tumour progression are of particular interest. There are two major goals. The first goal is delineating the signalling mechanisms between normal melanocytes and keratinocytes. Cadherins are the most likely candidates for signal transfer between these cells. However, whether the catenin system is being utilized or whether a co-receptor and its downstream signals are active warrants further investigations. The second goal is developing strategies to repress melanoma cells by keratinocytes. Potentially, the regulation of this process occurs through master genes. Their identification should help to develop rational strategies for therapies that result in tumour stasis.

The functional inactivity of the ETRB and *c-kit* pathways both appear to be critical for melanoma cell survival. Elucidating the mechanisms of down-regulation of these pathways should be major research goals in the melanoma field.

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