

Cell Lineage and Cell Migration in the Neural Crest

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A major problem facing investigators of tuberous sclerosis (TSC) is the need to explain the diverse and heterogeneous phenotypes of affected people with a single chromosomal mutation. This is compounded by attempts to reconcile aberrations within the central nervous system (CNS) with those scattered throughout the remainder of the body. The neural crest, a single cell population that forms or interacts with most of the tissues affected by the TSC mutation, could bridge this gap between the chromosomal alteration and cellular manifestation of TSC. Considering TSC as a neural crest disorder may simplify models for the cellular mechanism underlying this disease.

ORIGINS OF THE NEURAL CREST

The neural crest is a transient embryonic structure whose cells have been widely studied because of their large number of cellular derivatives and their ability to migrate as single cells throughout the embryo. The neural crest forms at the apex of the elevating neural folds at the onset of neurulation. As neurulation continues, the neural tube closes and the neural crest cells come to lie on the dorsal-most portion of the neural tube in avian embryos. Neural crest cells emigrate from the neural tube, migrate extensively, and differentiate in a region-specific manner, forming the peripheral nervous system, facial cartilage and bone, chromaffin cells of the adrenal medulla, and melanocytes.

The neural crest has been divided operationally by the axial level from which the cells originate into cranial, vagal, and trunk neural crest. These regional divisions have functional relevance. The cranial neural crest gives rise to the neurons and glia of the cranial ganglia as well as to facial cartilage and bone. Trunk neural crest gives rise to the neurons and glia of the sensory and sympathetic ganglia, melanocytes, and chromaffin cells of the adrenal medulla. Vagal crest cells migrate caudally to give rise to the neurons and glia of the enteric ganglia. The intrinsic nature of this regional specificity has been explored in a series of experiments in which neural tubes containing the premigratory cranial neural crest cells from quail embryos were transplanted into the trunk region of a developing chick and *vice versa*.¹ In these experiments, quail and chick cells could be identified by their distinct nucleolar morphology. Some cranial neural crest cells were able to form trunk neural crest derivatives, although other cells formed cartilage, a derivative normally only found in the head. Conversely, trunk crest cells failed to form the cartilaginous derivatives when transplanted into the head. Hence, neural crest cells from different axial levels have some restriction in

their differentiation before emigration from the neural tube, but they are partially flexible in their prospective fate.

One basic question posed by researchers investigating the neural crest concerns how this apparently homogeneous population of cells can differentiate in a region-specific manner to form a large variety of cell types. Two basic determinants underlying the control of neural crest differentiation, environmental and intrinsic, are presently under investigation.

ENVIRONMENTAL REGULATION OF NEURAL CREST CELL MIGRATION

Environmental cues may be important for the regulation of neural crest cell migration and differentiation because of the variety of migration pathways taken by these cells before their regional dependent differentiation. Neural crest cells could obtain external cues for migration from three possible sources: cell-extra-cellular matrix and cell-cell interactions in their local environment, and diffusible signals, which may allow distant tissues to influence the neural crest. Within these groups, at least three possible signaling mechanisms exist. Causal or instructive signals may direct neural crest cells by telling them where to migrate and how to subsequently differentiate. This would be analogous to a well-marked highway, where automobiles navigate by instructive road signs. Permissive signals may allow neural crest cells to migrate through a given area without imparting information to the cells in the form of either directional cues or signals that support differentiation. This would be an unmarked highway by analogy. Inhibitory signals may prevent neural crest cells from migrating through a given area of the embryo or may limit their differentiative potential. In our extended analogy, they may work as a roadblock.

To understand how neural crest cell migration is regulated, the precise pathways followed by migrating neural crest cells must be determined. This discussion is limited to the pathways of migration in the developing avian embryo inasmuch as these have been most extensively studied¹⁻¹⁰ because of their ease of manipulation and the availability of good cellular and extracellular matrix (ECM) markers. Less is known about mammalian and amphibian neural crest cell migration, although these organisms have been receiving more attention in recent studies.¹¹⁻¹⁵ The pathways of neural crest cell migration have been mapped using the monoclonal antibody HNK-1, which recognizes a surface carbohydrate present on avian and rat neural crest cells.^{3,5,6,7,13} Neural crest cells begin their migration in or near the dorsal aspect of the neural tube or neural folds. In the trunk of the developing embryo, neural crest cells migrate laterally and ventrally away from the dorsal aspect of the neural tube toward the developing somite. As they exit the neural tube, they initially encounter a cell-free space filled with ECM molecules. The first cellular structure that neural crest cells encounter is the developing somite, where they migrate ventrally through the sclerotomal portion of the somite. In serial sections, neural crest cells apparently do not migrate through all parts of the somite uniformly, but they migrate in a metameric pattern.^{4,5} Neural crest cells migrate through the rostral sclerotome, but not the caudal sclerotome. Consequently, neural crest cells are thought to be either guided to the rostral sclerotome or prevented from entering the caudal sclerotome.

A number of cell surface and ECM molecules have been studied to see if they could contribute to this metameric distribution of neural crest cells. Some mole-

cules, like fibronectin and laminin, are uniformly distributed throughout the sclerotome during the period of neural crest cell migration.¹⁶⁻²⁰ Others, like tenascin²¹⁻²⁵ and collagen type III,²⁶ show a metameric distribution after neural crest cells enter the sclerotome, but not before. A few molecules, such as T-cadherin and peanut lectin binding molecule(s), are present in a metameric pattern before and during neural crest cell migration. T-cadherin is present within the caudal sclerotome before neural crest migration into the rostral sclerotome.²⁷ Ablation of the neural crest does not alter the metameric pattern of T-cadherin expression.²⁷ In contrast, ablation of the neural crest does alter the metameric pattern of expression of tenascin or collagen type III, indicating that their distribution is affected by migrating neural crest cells.²⁵ The expression of molecules like T-cadherin within the caudal sclerotome has led to the hypothesis that inhibitory molecules may play a significant role in controlling the migration of neural crest cells. As neural crest cells migrate ventrally, they fail to penetrate a region of about 100 μm around the notochord.²⁸ This has led to the speculation that inhibitory molecules, like chondroitin sulfate proteoglycans present within the perinotochordal region, are responsible for inhibiting neural crest cell invasion of the area.

The correlation of the distribution of a molecule with the pathways of neural crest cell migration is insufficient to determine whether the molecule plays any role in the regulation of neural crest cell migration. Functional studies of cell-ECM interactions are necessary in order to determine a molecular importance. Several functional perturbation experiments have been useful in examining the role of the ECM in neural crest cell migration. These include (1) the use of antibodies and peptides that functionally block cell-ECM interaction within the embryo; and (2) the use of function-blocking antibodies and peptides on cultured neural crest cells grown on purified ECM molecules. The former determines whether the interaction blocked by the antibody or peptide is important to normal neural crest cell migration, whereas the latter is useful in determining the specificity of the perturbation and the molecular basis of cell-ECM interaction. A number of ECM molecules have been implicated in the control of neural crest cell migration. Molecules like laminin,^{18,19} fibronectin,^{16-18,20} and integrins,¹⁸ a family of cell surface receptors for ECM molecules,²⁹⁻³¹ are present along the pathways of neural crest cell migration and are therefore good candidates for regulating their migration.

***IN VIVO* PERTURBATION OF NEURAL CREST CELL MIGRATION**

In the cranial region of embryos, neural crest cells migrate along the subepidermal basement membrane and between the adjacent mesenchymal cells. To test for the functional importance of cell-ECM interaction within the embryo, Marianne Bronner-Fraser^{29,32-35} and others³⁶ have employed a technique whereby molecules could be injected into the cranial mesenchyme in an attempt to perturb the migration of neural crest cells. One advantage of this technique is that antibodies generally do not cross the midline of the embryo, allowing the side of the embryo contralateral to the site of injection to act as a control for the experimental side. In animals injected with function-blocking antibodies against ECM receptors or ECM molecules, several abnormalities in normal neural crest cell migration were observed, including; (1) large numbers of neural crest cells that failed to leave the neural tube and remained in the dorsal portion of the neural tube long

after emigration had ceased, (2) reduced numbers of neural crest cells compared with the control side, and (3) neural tube defects.

The results of a number of these perturbation studies are summarized in TABLE 1. Multiple function-blocking antibodies directed against cell surface components involved in cell adhesion and cell-matrix interactions cause changes in the normal migration of cranial neural crest cells, whereas nonfunction-blocking antibodies had little or no effect. In contrast, none of these antibodies has had any effect on neural crest cell migration in the trunk of the embryo. This indicates that the trunk neural crest cells or their environment is different from the cranial neural crest or that this technique does not deliver sufficient quantities of the antibodies to the trunk regions.

ECM molecules have also been shown to influence amphibian neural crest cell migration.³⁷ Complex ECM was transplanted from embryos where neural crest migration is well advanced into the prospective neural crest migratory pathways

TABLE 1. Results of *in Vivo* Perturbation of Avian Neural Crest Cell Migration

Ref.	Antibody	Affected Embryos	Affected Regions		NT Abnormality	NC Reduction
			NC in Lumen	Ectopic NC Cells		
35	INO	+++	+	++	+	-
33	CSAT	+++	+	+	+	++
22	Anti-TN	+++	+	+++	++	-
34	HNK-1	+++	++	++	+	-
34	CSAT	+++	++	-	-	+++
34	HNK-1 + CSAT	+++	+	++	+	+
	Anti-N-CAM ^a	++	++	++	++	-
	Anti-N-cadherin ^a	++	++	++	++	-
22	Anti-FN ^b	+	+	+	+	-
22	Anti-LM ^b	+	+	-	-	-
22	Anti-N-CAM ^b	+	-	+	-	-

ABBREVIATIONS: CSAT = antibody to B₁ subunit of integrin; FN = fibronectin; HNK-1 = antibody to carbohydrate epitope on neural crest cell surface; INO = antibody to a laminin-heparan sulfate proteoglycan complex; LM = laminin; NC = neural crest; N-CAM = neural cell adhesion molecule; NT = neural tube; TN = tenascin.

^a Data from unpublished results of Dr. Marianne Bronner-Fraser.

^b Nonfunction blocking antibodies.

of younger embryos. This complex ECM caused the precocious migration of neural crest cells. These studies indicate that the ECM can influence neural crest cell migration directly.

IN VITRO NEURAL CREST CELL INTERACTIONS WITH THE ECM

Another advantage of the neural crest as an experimental system is the relative ease with which a pure population of cells can be obtained. Neural tubes isolated before neural crest cell migration can be placed on a suitable substrate (like fibronectin) in a tissue culture dish. After several hours, the neural crest cells migrate away from the neural tubes, which can then be removed to yield a rela-

tively pure population of cells. This has led to a large number of studies demonstrating that a variety of ECM molecules, such as fibronectin, laminin, and collagens, are permissive for neural crest cell migration *in vitro*.³⁸⁻⁵⁵ Integrin receptors on the surface of neural crest cells mediate the interactions of these cells with specific ECM molecules. When neural crest cells grown on a fibronectin substrate are incubated with a function-blocking antibody for β_1 subunit of integrin, they detach from the substrate.³² This demonstrates that antibodies that affect neural crest migration *in vivo* can be used *in vitro* to further define their interaction with neural crest cells.

The assays for neural crest cell migration *in vitro* are difficult to quantify. Another more quantitative type of assay is one that measures cell-matrix adhesion directly.⁵⁴ In these studies, radioactively labeled neural crest cells are brought into contact with an extracellular matrix molecule by centrifugation. After the cells have attached, nonadherent cells are removed with a known force by centrifugation, and the percentage of adherent cells can easily be calculated. From these experiments, the following model for neural crest cell-laminin interactions has been developed. At high concentrations of laminin, neural crest cell adhesion is dependent upon β_1 -containing integrins that require Ca^{2+} , but not Mg^{2+} . At low concentrations of laminin, neural crest cell adhesion is dependent upon β_1 -containing integrins that do not require divalent cations (contrary to all reported integrins) and either bear the HNK-1 carbohydrate or are associated with a molecule that does. Interestingly, *in vitro* experiments measuring the extent of neural crest cell migration on planar laminin substrates⁵³ demonstrate greater migration at low laminin concentrations than at high laminin concentrations. This implies that different neural crest cell surface receptors for laminin may play different roles in their migration. Neural crest cell adhesion fibronectin and collagens are mediated by RGD-dependent integrins.⁵³

CELL-CELL INTERACTIONS

Cell-cell adhesion has been proposed to be important for aspects of neural crest cell development, particularly in ganglion formation. The neural cell adhesion molecule (N-CAM) is present on neural crest cells before their emigration from the neural tube and within the dorsal root ganglion after its formation.⁵⁶ However, when neural crest cells first begin to aggregate to form the dorsal root ganglia, N-CAM is not expressed within the ganglia.⁵⁷ By comparison, the temporal expression of N-cadherin in the forming dorsal root ganglia occurs before ganglia formation (Tatsuo Akitya, personal communication). In a graphic representation of the levels of apparent expression of these two cell adhesion molecules (FIG. 1), we see that neural crest cells express both N-CAM and N-cadherin before migration, lose both molecules during emigration from the neural tube, and express little or none while migrating. In the case of the dorsal root ganglia, neural crest cells begin to express N-cadherin at the time of or before ganglia condensation, with N-CAM expression rising after the ganglia have condensed. This implies that N-cadherin, but not N-CAM, may be involved in the aggregation of neural crest cells to form the dorsal root ganglia. It is interesting that in the sympathetic ganglia, also derived from the neural crest, the rise in N-CAM expression is coincident with ganglion formation,^{57,58} indicating that different neural crest cell derivatives may use different cellular mechanisms to perform essentially the same tasks.

CELL LINEAGE CONTROL OF NEURAL CREST CELL DIFFERENTIATION

In addition to environmental factors, neural crest cells may possess some intrinsic information that could control their differentiation and migration. This information could be in the form of decisions programmed into these cells before their emigration from the neural tube. This program of cell fate can be divided into (1) instructions for multipotent cells to later differentiate into specific cell types, a subset of which could be the sequential limiting of cell potential, (2) the predetermination of cell fate with subsequent selection for a limited population of cells, or (3) a combination of instructive and selective decisions.

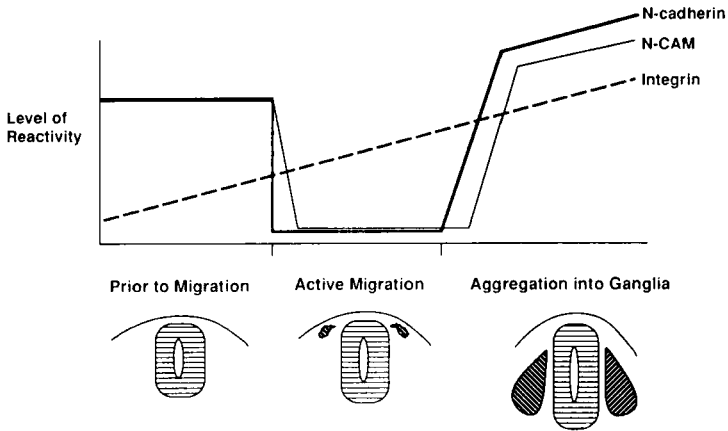


FIGURE 1. Cell adhesion molecules in neural crest development. Relative levels of N-CAM (neural cell adhesion molecule), N-cadherin (neural calcium-dependent cell adhesion molecule), and integrin (extracellular matrix receptors) on neural crest cells detected immunohistochemically. Cell-cell interactions appear to predominate before migration and after aggregation of the dorsal root ganglia. Cell-matrix interactions appear to predominate during neural crest cell migration. N-cadherin expression coincides with ganglia aggregation, indicating a possible role in gangliogenesis. N-CAM expression follows after the initial aggregation of the ganglia, possibly reinforcing the cell-cell interactions initiated by N-cadherin.

The potential cell fates of neural crest cells with respect to their lineage have been the focus recently of studies performed by Bronner-Fraser and Fraser.^{59,60} In these studies, the lineage of neural crest cells was investigated to see if cells of a specific derivative (i.e., the dorsal root ganglia) were clonally related or if a single neural crest cell could give rise to a number of cell types and therefore be multipotential. These studies into cell lineage in neural crest cells were performed by injecting cell lineage tracer dyes (lysinated rhodamine dextran) into a single cell in the dorsal aspect of the neural tube before the onset of neural crest cell migration. The dye used in these experiments has the advantages that it will not pass from one cell to another, except by cell division, and it can be detected after several cell divisions. The results of these experiments showed that almost all possible combinations of clonally related derivatives can arise from individual neural crest cells.

This indicates that some neural crest cells are multipotent and may be influenced to differentiate by environmental cues. These results hold true for neural crest cells whether injected before migration, during emigration from the neural tube, or during their migration through the somite. Single clones were able to give rise to neurons in multiple neural crest derivatives, whereas some neural crest cells shared common progenitors with neural tube cells.

POTENTIAL LINKS BETWEEN THE NEURAL CREST AND TUBEROUS SCLEROSIS

If the neural crest and its derivatives were the only cell type affected by the TSC mutation, a large number of TSC symptoms could be explained. This section will be speculative by necessity, because studies into the behavior of early human neural crest cells are impossible to perform and, to date, no animal model system for TSC exists.

To relate TSC phenotypes to the neural crest, we will begin with the CNS aberration of subependymal nodules. In the *in situ* perturbation experiments of Bronner-Fraser^{29,32-34} neural crest cells of cranial origin, if functionally blocked from interacting with the ECM or other cells, failed to migrate normally, with some cells moving into the lumen of the mesencephalic neural tube. In these studies, some abnormalities were overcome by the regulative ability of the embryo, yielding relatively normal embryos 2 days after antibody injection, whereas other abnormalities persisted for some time. This could be explained easily by the degradation of the antibody, possibly by cellular internalization of the affected receptors or by protease activity within the matrix. Thus, these experiments provide the equivalent of short-term functional mutation in a limited region of the embryo. Possibly, a similar scenario exists in TSC, resulting in cranial neural crest cells migrating into the cephalic ventricles. In TSC this would not be a temporary perturbation, and these cells might survive for some time within this environment. Le Douarin¹ and colleagues demonstrated that some cranial neural crest cells, when placed in an abnormal environment, retain their ability to differentiate into cartilage. This was demonstrated by placing cranial neural crest cells into the pathways followed by trunk neural crest cells. The neural crest cells that give rise to cartilage normally develop into the facial bones. Accordingly, some cranial neural crest cells that migrate inappropriately into the cephalic ventricle could conceivably differentiate into cartilage, which could later give rise to calcified cell aggregates. Thus, calcified subependymal nodules found in patients with TSC may result from misplaced neural crest cells. Consistent with this possibility, neural crest cells grafted under the kidney capsule form teratomas containing cartilage and bone.⁶¹

The hypomelanotic macules of patients with TSC involved an alteration in melanocytes, a cellular derivative of the neural crest. From descriptions of these "white patches," melanocytes appear to be present, but individual melanocytes have reduced production of melanin. This implies that the melanocytes apparently migrate "normally" to their destination, although their subsequent differentiation is affected by the TSC mutation. If the tissues normally populated by the neural crest are examined and compared with the abnormal tissues found in patients with TSC, a striking similarity is apparent (TABLE 2). Although not all TSC affected tissues are of neural crest cell origin, many interact with the neural crest cells during their development.

The human genetic disorder, retinoblastoma (RB), has been shown to be the result of two mutations, as originally proposed by Knudsen.^{62,63} A discussion of a similar "two-hit" mutation model will be presented in some detail by W. Johnson elsewhere within this volume. In the two-hit model as it applies to RB, an inherited mutation in one allele for a gene increases the likelihood of a subsequent somatic cell mutation of the same gene, manifesting in a cellular disorder. In RB, everyone has two copies of the RB gene. People with the familial form of the disease have a mutation in one of these genes. As the cells of their bodies divide during development, occasional mutations occur that are not repaired, such as

TABLE 2. Comparison of Neural Crest Cell Derivatives with Tissues Affected by Tuberous Sclerosis

TSC Affected Tissues	Neural Crest Derivative
<i>CNS</i>	
Subependymal nodules	Facial cartilage
Intraventricular astrocytomas	
Giant-cell astrocytomas	Cranial ganglia
Cortical tubers	
<i>Eye</i>	
Retinal hamartomas	Ciliary ganglia
<i>Teeth</i>	
Dental pits	Dentine
	Enamel ^a
<i>Skin</i>	
Hypomelanotic macules	Melanocytes
Facial angiofibroma	Cranial mesenchyme
Ungual fibromas	
Shagreen patches	
<i>Kidney</i>	
Angiomyolipomas	Chromaffin cells
Renal cysts	
<i>Heart</i>	
Rhabdomyoma	Aorticopulmonary septum of the truncus arteriosus
	Cardiac ganglia
Great vessels	Smooth aortic muscle
<i>Gastrointestinal</i>	
Rectal polyps	Enteric ganglia
<i>Unaffected tissues</i>	
	Sensory ganglia
	Sympathetic ganglia

^a Enamel is thought to result from a neural crest cell inductive event.

somatic cell chromosomal translocations. Subsequently, this cell carries two mutations, one in each of its RB genes, leading to individual tumors. A limited number of RB tumors exist within an individual because the retinal cells are particularly sensitive to RB mutations, and the retinal cells are not a continuously replenishing cell population. This leads to the conclusion that the RB gene is critical to the control of retinal cell differentiation. RB, like TSC, has disorders that can be subdivided into familial and sporadic forms of the disease.

If we apply a similar model directly to the neural crest, we can produce a plausible explanation for the cause of TSC. A two-hit model for TSC, with the

neural crest as the sensitive tissue, could have a larger number of tumors than those found in patients with RB and a greater diversity of affected tissues because of the migration and multipotentiality of neural crest cells. For example, if one "premelanocyte" neural crest cell received its two hits causing it to become a $TSC^{(-/-)}$ cell, it might still possess its ability to divide and migrate. Therefore, this cell could migrate and intermingle with "normal" ($TSC^{(+/-)}$) melanocytes and spread throughout the body. At some as yet unknown time when melanocyte migration halts in humans, these cells would then divide to become a patch of clonally derived "white" cells. As a consequence, the earlier the "second hit" occurs in development, the larger will be the predicted number of $TSC^{(-/-)}$ affected cell clusters. If human premelanocyte migration mimics that in avians¹⁰ and mice¹⁵ temporally, we would expect that premelanocytes are some of the last neural crest cells to migrate away from the neural tube. Therefore, we could expect to find people who have only one or a few white patches, without having any other symptoms of TSC due to a very late second hit, and who still have TSC. This person could have the same propensity to pass on the TSC first hit to his/her children as would a patient with a larger number of symptoms of TSC.

The multipotentiality of the neural crest^{59,60} also helps to explain the variety of symptoms of TSC within the context of this model. As shown in these studies, a given neural crest cell could give rise to any number of cellular derivatives scattered throughout the body. Consequently, if a cell that would give rise to melanocytes and chromaffin cells of the adrenal medulla received its second hit, the progeny of this cell could form aberrant cell patches of melanocytes and kidney nodules. Therefore, it would not be unexpected to have one or two clonal clusters of $TSC^{(-/-)}$ cells in a variety of organs. The heterogeneity of phenotypes found in neural crest cell clones shows no predictable frequencies, a parallel seen in the symptoms of patients with TSC. As stated earlier, one prediction of this model is that the earlier in development the "second hit" occurs, the larger will be the number of predicted $TSC^{(-/-)}$ cell clusters and the greater their potential diversity. This could be reduced by any restrictions possessed by the particular clonal phenotype of the original $TSC^{(+/-)}$ cell(s) (i.e., late hits in cells of neuronal lineages may produce fewer $TSC^{(-/-)}$ clusters than may late hits in melanocyte lineages).

Models describing how neural crest cells interact with their environment and thereby receive cues for their guidance and differentiation involve the ability of these cells to recognize several of the ECM and cell-surface molecules they encounter. As neural crest cells migrate through their environment, they could be directed to change their cell surface receptors for ECM or cell adhesion molecules. This is reflected in the observation that neural crest cells lose N-CAM and N-cadherin from their surfaces during migration and later regain them during gangliogenesis. This differential preference for cell surface and ECM components is proposed to be important in neural crest cell guidance and differentiation. If $TSC^{(-/-)}$ cells have this preference altered by the loss of some cell surface receptor, their guidance and subsequent differentiation could be altered. Cells that normally migrate along one substrate could ignore it and migrate into an entirely different region of the embryo and once there differentiate into the same or a related cell type (FIG. 2). Alterations in the protein products of $TSC^{(-/-)}$ cells, when compared to those of "normal" cells, could be the result of aberrant differentiation, rather than direct alteration of the genes encoding for these proteins. This could lead to precocious migration or differentiation of the affected cells in a manner similar to that seen when neural crest cells are exposed prematurely to a migration-promoting matrix.³⁷ In neural crest cells, this could alter their path of

migration. If neural crest cells differentiate as they migrate, this could alter their substrate preference and cause cells to migrate into regions where they are not normally found.

The ability of mutations in multiple genes, possibly found on different chromosomes, to have similar effects on neural crest cell migration is not unknown. In comparing a variety of *in vivo* perturbation experiments,^{29,32-34} we found that perturbation of several cell-ECM interactions (e.g., integrins, laminin, and tenascin) leads to similar disruption of neural crest cell migration. Perturbation of cell-cell interactions yields similar abnormalities. Therefore, the proper migration of cranial neural crest cells seems to depend on a variety of cell surface interactions, where the perturbation of any one is sufficient to alter normal cell migration. In TSC this could reflect a two-hit alteration in any of several genes vital for normal development, which result in roughly similar phenotypes.

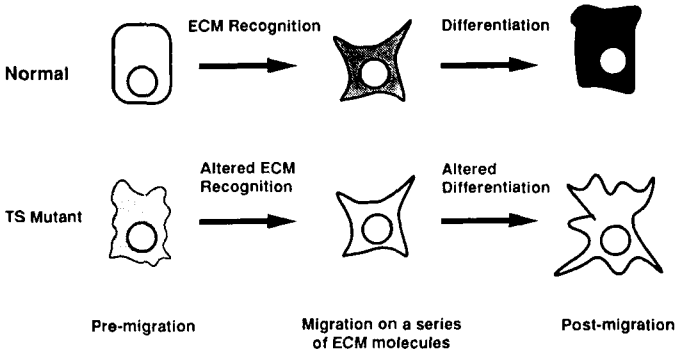


FIGURE 2. A model for tuberous sclerosis mutation effects on neural crest cell migration. Normal neural crest cells may migrate through a series of extracellular matrix molecules, causing them to differentiate into a specific cell type. Tuberous sclerosis mutant cells may lack the ability to properly interact with the matrix molecules they encounter, leading to misplaced cells or cells that differentiate into abnormal cell types. ECM = extracellular matrix; TS = tuberous sclerosis.

A two-hit model for TSC also helps to explain the sporadic cases of TSC, as it has helped to explain the sporadic cases of RB. Sporadic cases of TSC are those that appear suddenly, with no previous symptoms found in preceding generations. With this model, sporadic cases would be predicted to arise when two independent hits occur during the development of an individual. If the first hit occurs before the segregation of the germ-line cells, the individual would possess a chimeric germ line. Such patients would pass on the TSC mutation to subsequent generations with a probability of less than 50%. If such patients exist, their offspring would be predicted to express TSC with a lower frequency than would the offspring of patients with familial TSC. If the germ line segregated before the first hit occurred, a patient with sporadic TSC would have the same probability of having TSC^(-/-) offspring as would any "normal" individual, or about 1 in 15,000.

The two-hit model for TSC can be summarized as follows:

1. TSC requires the alteration of at least two genes (or alleles of the same gene) before symptomatic aberrations become apparent.

2. The neural crest is either the only sensitive or the most sensitive tissue to the occurrence of the second hit or the product of the second hit.

3. The second hit probably alters the differentiation of neural crest cells. This could be the result of alterations in their migration pathway and inductive signal. This model for TSC, which intimately involves the neural crest cells, is highly speculative, but it may lead to increased interest in the cellular mechanisms underlying TSC and a better understanding of how this genetic disorder can involve mutations on multiple chromosomes that lead to a variety of related phenotypes.

SUMMARY

The neural crest is a transient embryonic structure whose cells migrate extensively before giving rise to a variety of differentiated cell types. Both intrinsic cell lineage information and environmental cues are thought to play a role in determining the fate of these cells. Early in development, these cells can be divided into distinct populations based on their axial level of origin. Cranial neural crest cells differentiate into facial cartilage and cranial ganglia, whereas vagal crest cells give rise to the enteric ganglia. Trunk neural crest cells normally give rise to melanocytes, neurons, and glia of the peripheral nervous system and chromaffin cells of the adrenal medulla. Cell lineage studies of premigratory trunk neural crest cells using single cell injection of a vital dye have shown that single cells can give rise to a number of differentiated cell types. A host of extracellular matrix (ECM) molecules have been tested for their ability to support neural crest cell migration *in vitro* and *in vivo*. In general, the large glycoproteins (i.e., fibronectin and laminin) can support migration, whereas proteoglycans seem to modulate neural crest cell migration on other ECM molecules. However, no single molecule has been identified as the sole regulator of the complex pattern of neural crest cell migration.

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