

# Insight into Neurodegenerative Disorder Using Melanocytes as a Model System

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## Abstract

**Background:** Neural crest cells (NCCs) by responding to several signals and paracrine factors get differentiated into different lineages like peripheral nervous system (PNS), chondrocytes, myofibroblast, endocrine, melanocytes, etc., Melanocytes are pigment-producing cells that share a common origin, paracrine factors (Wnt, FGF, and BMP), and transcription factors (TFs) with the neurons of the nervous system. **Objective:** Neuronal model for neurodegenerative disorders are limited because of their nonhuman origin and transformation. In this review we propose the use melanocyte as a model system to study neurodegenerative studies. **Method:** Systematic Literature Review. **Results:** The similarity between neural crest-derived melanocytes and neurons, makes melanocyte an important model to study several neurodegenerative disorders like Alzheimer's disease and Parkinson's disorder. **Conclusion:** Melanocytes and neurons share common origin i.e. both arise from NCC and share identical signalling molecules and pathways. Neural crest-derived melanocytes can thus serve as a promising model system to study normal and pathological behaviour of less accessible neurons.

**Keywords:** Melanogenesis, neural crest, neurodegenerative disease, transcription

## INTRODUCTION

Stem cells like neural crest cells (NCCs) are the specialized cells that have the capacity of self-renewal and form different lineages of cells. Neural crest formation requires both inducer (ectoderm and paraxial mesoderm) and competent cells (neural plate). Differentiation into specific fate depends upon extrinsic as well as intrinsic signaling. Signals like bone morphogenetic proteins (BMP), fibroblast growth factors (FGF), and Wnt pathways, which are secreted by paraxial mesoderm, are involved in the induction of neural crest. The gene regulatory network (GRN) guides several signaling pathways and the transcription factor (TF) to acquire specific properties, such as multipotency and migration. Figure 1 summaries the gene regulatory networks required for neural plate induction and NCC formation.<sup>[1]</sup>

Terminal differentiation of neural crest populations depends upon the migration pathway they follow and signals generated by the neighboring cells. As shown in the Figure 2.<sup>[2]</sup> During the course of migration, melanocyte precursors migrate dorsoventrally to reach skin and hair follicles, whereas, neuronal precursors migrate to the brain and other peripheral tissues.

The development of both melanocytes and neurons depend upon the signal generated by neighboring cells. Signaling pathways that play important role in the development of the central nervous system (CNS) and peripheral nervous system (PNS) also have a role in generation of pigment cells. Due to these similarities between melanocytes and neuron,

melanocytes can be used as an in-vitro model to study normal and pathology of disorders that affect the nervous system.<sup>[1,3-5]</sup>

## Transcription factors and signals associated with differentiation of neural crest cells into different lineages

Although NCC are pluripotent, cells originated from different anteroposterior regions are different. Table 1 shows the different NCC, their migration and the fate they acquire.<sup>[6]</sup> The development of NCC into different lineages depends upon the balance between these extrinsic and intrinsic signaling as shown in Figure 3.<sup>[2,7-11]</sup>

Forkhead Box D3 (FoxD3) is an important TF that is critical for NCC migration, and it regulates lineage switch between neuronal cells and melanocytes. In neuronal cells bone morphogenetic proteins (BMP) activates the expression of FoxD3, which further inhibits the expression of microphthalmia-associated

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**Table 1: NCC migratory pathways and its association with cellular fate**

Types of NCCs	Derivatives
Cranial (cephalic) NCCs	Cells migrate dorsoventrally to form craniofacial mesenchyme (cartilage, bone, glia, cranial neurons, and connective tissue).
Trunk NCCs	Early cells migrate ventrolateral and form sympathetic ganglia, adrenal medulla, and nerve cluster surrounding aorta. Later migrating cells form melanocytes.
Vagal and sacral NCCs	They form parasympathetic ganglia of the gut.
Cardiac NCCs	They can develop into melanocytes, neurons, and cartilage of connective tissue.

NCC=Neural crest cell

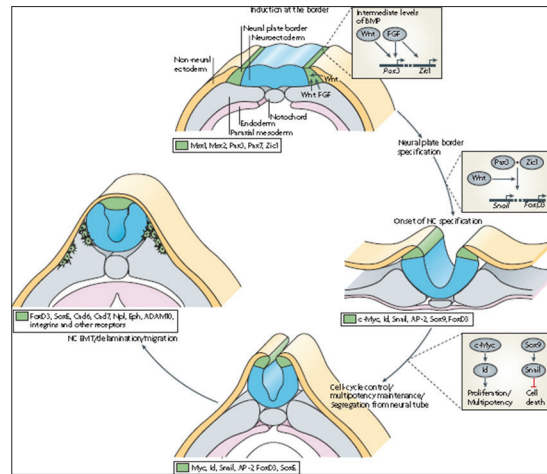
transcription factor (MITF). MITF functions as the master regulator that is involved in the development as well as survival of melanocyte.<sup>[2,6,11-13]</sup> Cells that migrate early express FoxD3 in conjunction with other MITF-inducing factors. FoxD3 interacts with paired box 3 (Pax3) and SRY-box 10 (Sox10) and inhibits their binding to MITF promoter. Hence, it inhibits the process of melanogenesis in the cells that are specified to become neurons.<sup>[1,3,13-18]</sup>

Sox10 function's as a central transcription regulator of neural crest development. It is expressed in the neural crest at the time of migration and its expression continues in the differentiated cells. Both Pax3 and Sox10 interact synergistically and regulate survival as well as differentiation of NCC.<sup>[1,3,18]</sup>

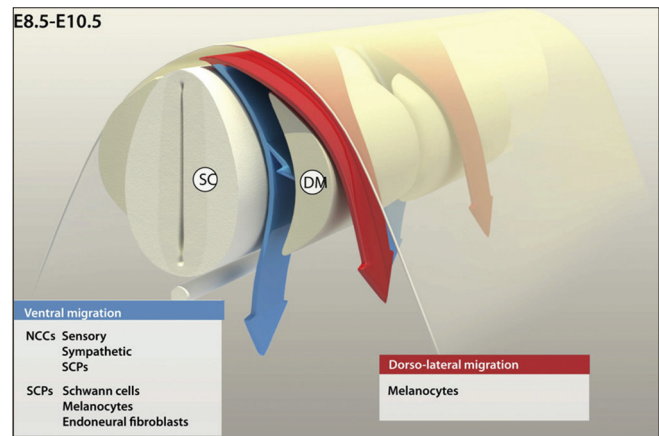
Neurons and melanocytes share various signaling molecules that play an important role in their proliferation, survival, maintenance, and migration. Wnt signaling regulates neural crest induction and differentiation during neural crest development. When activated in premigratory NCC, it promotes the formation of neurons, whereas suppresses melanocyte formation. In the nervous system, it leads to the patterning of CNS and regulates neuronal growth and survival. Cells which migrate dorsoventrally form melanocytes by activating the Wnt pathway. In melanocyte, Wnt pathway activates the expression of transcription factor, microphthalmia-associated transcription factor (MITF). MITF is the master regulator, which regulates the expression of many melanogenic enzymes important in melanin formation.<sup>[5,11,15]</sup>

FGFs are membrane-bound factors which are expressed by keratinocytes that surround melanocytes and fibroblasts. These FGFs through cell-cell interaction activate proliferation of melanocytes and also act as a potent melanocyte mitogen that functions as a cAMP stimulator. FGF1 and FGF2 play important roles in proliferation, differentiation, axonal guidance, and survival of cells of the nervous system.<sup>[6]</sup>

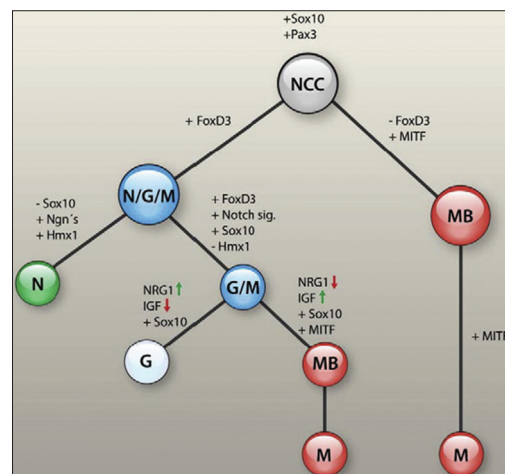
Neurotrophin (NT) includes small molecules like nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) that have important roles in neuronal survival by binding to two receptors named, p75NTR (low-affinity receptor) and



**Figure 1:** Regulatory steps in neural crest formation (Spengler *et al.* Nature Review, 2008)



**Figure 2:** Illustration of the NCC migratory pathways and its association with cellular fate. The main cell types of NCCs migrating in the ventral migratory pathway include sensory, sympathetic and SCPs. SCPs are the cellular source for Schwann cells, melanocytes and endoneurial fibroblasts. SC, spinal cord; DM, dermomyotome; NCCs, neural crest-derived cell types (Figure taken from Ernfor's *et al.* Experimental Cell Research, 2010)



**Figure 3:** Transcription factors and signals involve in neural crest cell differentiation (Figure taken from Ernfor's *et al.* Experimental Cell Research, 2010)

Trk (high-affinity receptor).<sup>[4,5,16]</sup> Melanocytes express p75<sup>NTR</sup> and TrkA as well as NT3 that are high-affinity receptor. During ultraviolet (UV) irradiation, keratinocyte (KC) start secreting NGF, which act as a chemotactic signal for melanocyte by inducing dendrite formation and also increases Bcl2 (anti-apoptotic protein). Hence, coordinated expression of p75<sup>NTR</sup> and TrkA and their binding to NGF and NT3 cytokines released by KCs increases melanocyte survival after UV irradiation. Similarly, function is performed in the nervous system and enhances neuronal survival in CNS as well as in PNS. BDNF plays an important role in the survival of motor neurons by binding to high-affinity TrkB receptor. In the adult nervous system, they also regulate synaptic plasticity and neuronal survival.<sup>[5,9,19]</sup>

Many signaling molecules are involved in the migration of melanocytes and neurons to their final destination. Steel factor is one of them. Kit ligand expressed by the keratinocytes and c-kit receptor is present on melanocyte. As soon as the receptor is expressed by melanocytes and kit ligand by KCs they start migrating towards skin and hair follicles.<sup>[16,18]</sup> Steel factor and c-kit are also expressed in adult CNS, specifically hippocampus.

Endothelin's (ET) formed by proteolysis of larger precursor molecule characterized by their vasoactive properties. This family of proteins includes ET1, ET2, and ET3. ET1 binds to heptahelical EdnrA receptor whereas, ET3 binds to EdnrB and regulate survival, migration, migration and also has photoprotective effects. These receptors are present in melanocytes and neuronal cells. During UV irradiation, ET1 synthesized and secreted from keratinocytes, binds to EdnrA on melanocyte and induces photoprotective responses. Similarly, in brain exposure to neurotoxic agents increases the level of these peptides and hence protection of neurons.<sup>[5,6]</sup>

Signaling molecules activate downstream pathways by binding to their receptor. Two major signaling pathways shared by melanocytes and neurons are:

#### *Protein kinase C (PKC) –dependent pathway*

Receptor such as EdnrA, EdnrB, Trk and FGF when get activated, leads to the formation of secondary messenger diacylglycerol which further activates PKC. Melanocytes express 5 isoforms of PKC to regulate melanin formation, survival after stress and dendrite formation. PKC  $\beta$  is mainly involved in regulating the activity of tyrosinase present in melanosome by phosphorylating it. During UV irradiation, Trk and Ednr gets activated to facilitate melanocyte survival (activation of anti-apoptotic factor Bcl2) and transfer of melanosome from melanocytes to keratinocytes by extending dendrites. This is mainly achieved through mediating cleavage of diacylglycerol and activation of PKC.

In the nervous system, PKC-dependent pathway gets activated during oxidative stress and helps in cell survival and regeneration.<sup>[5,12]</sup>

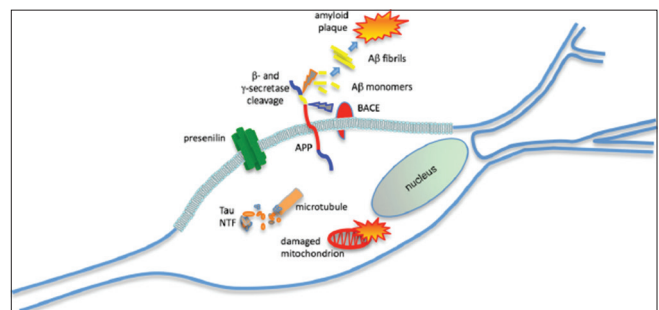
#### *p53-dependent pathway*

Tumor-suppressing protein p53 plays an important role during cellular stress. It regulates DNA damage repair, cell cycle arrest and apoptosis. UV irradiation both directly by DNA damage and indirectly through activators like H<sub>2</sub>O<sub>2</sub> activates p53. Activated p53 increases the process of melanogenesis by upregulating mRNA and protein of tyrosinase enzyme. p53 also activates transcription of proopiomelanocortin, an inducer of melanogenesis.<sup>[8,9,16]</sup> Posttranslational modification of p53 regulates the process of survival, differentiation and regeneration of nervous system. It plays an important role in neuronal regeneration after injury.

#### **Melanocytes as a model to understand Alzheimer's disease**

Alzheimer's disease (AD) is the most common neurodegenerative disorder affecting 35.6 million people worldwide. AD symptoms start with the loss of cognitive function and episodic memory due to the accumulation of amyloid plaques and neurofibrillary tangles. Amyloid plaques contain large amounts of a 42aa peptide called " $\beta$ -amyloid" or A $\beta$ 42 and neurofibrillary tangles are formed due to accumulation of cytoskeletal tau protein that gets heavily phosphorylated as shown in Figure 4.

Neurons of nervous system express many receptors p75<sup>NTR</sup> and Trk receptors are among them, that have role in neuronal survival during stress condition. Similar to neurons, melanocytes also express p75<sup>NTR</sup> which leads to melanocyte survival. Binding of NGF ligand to low affinity p75<sup>NTR</sup> receptor is through 29-30 aa residue (TDIKGKE). Studies showed that replacing K with A found that it still binds to the receptor but with little affinity.<sup>[5,10]</sup> Interestingly, in AD amyloid plaques which are formed by A $\beta$  also have toxic aa residue lying between 28-30 of sequence KGA. That means A $\beta$  can be a good ligand for p75<sup>NTR</sup> and induce apoptosis in AD patient. From, several in-vitro studies it has been shown that A $\beta$  specifically binds to p75<sup>NTR</sup> and induces apoptosis in both neurons and melanocytes. Hence, from this, it can be concluded that imbalance between p75<sup>NTR</sup> and Trk leads to age-associated accumulation of A $\beta$  in the brain causing loss of cholinergic neurons. There can be two possibilities which cause neuronal



**Figure 4:** Amyloid plaques and neurofibrillary tangles are the hallmarks of AD. Accumulation of these abnormal protein cause loss of cholinergic neurons and hence dementia (Figure taken from Crlo.Immunity and ageing 2012)

loss either p75 level goes up or extracellular level of A $\beta$  get increased. NGF levels are unaffected in AD patient. By doing immunohistochemistry with an antibody recognizing the Trk receptor, it has been shown that as compared to the normal individual, AD patient has a smaller number of neurons which express this receptor.<sup>[14,16]</sup>

## CONCLUSION

Several simple animal models such as worms, fishes, flies, ascidians and sea urchins are used to study the pathology of neurodegenerative disorders. Though, these model systems have played a very important role in the understanding of several biochemical mechanisms underlying AD neuronal model for neurodegenerative disorders are limited because of their nonhuman origin and transformation. So, for better understanding of nervous system functions and mechanisms of several neurodegenerative disorders like AD, we require a good model system. Melanocytes and neurons share common origin i.e. both arise from NCC and share identical signaling molecules and pathways. Neural crest-derived melanocytes can thus serve as a promising model system to study normal and pathological behavior of less accessible neurons.

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## Conflicts of interest

There are no conflicts of interest.

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