REVIEW ARTICLE

# Melanoma Redirects Schwann Cell Nerve Damage Repair Programming to Enhance Invasiveness

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**ABSTRACT**

The common outcome of interaction between solid tumors and the peripheral nervous system is neuropathic pain and possible extension of the tumor by means of perineural infiltration. This is not the case with melanoma. There are intra-tumor neural destruction and peritumoral attempted nerve repair. Axonal glial support cells are responsible for nerve repair. In the peripheral nervous system, the Schwann has this role. It has been found that melanoma cells can redirect Schwann cell nerve damage repair programming to enhance invasiveness.

**Key words:** Melanoma de-differentiation, regeneration invasiveness, schwann cell

## INTRODUCTION

The relatively high death rate of melanoma as compared to other skin cancers leaves no doubt as to its malignant potential. Genetic, epigenetic, and molecular factors have been intensively studied but this still does not fully explain differences in invasiveness and metastasis within the melanoma family. The melanoma microenvironment is increasingly recognized as being influential in this variability. To understand how melanoma is driven, we need to reference the study of neuroscience and the skin's response to nerve injury. Work on the pathogenesis of the Tasmanian devil's facial tumor disease has demonstrated that Schwann cell plasticity and immunocompetence may play a role in melanomagenesis augmenting invasiveness.[1] On melanoma invasion, the Schwann cell may revert to a progenitor-like cell with stem cell-like plasticity, encouraging a more invasive melanoma phenotype. In fact, plasticity is demonstrated by melanoma and Schwann cells as well as infiltrating macrophages.

Following nerve injury, neuronal degeneration alleviates pro-differentiation axonal signals triggering Schwann cell de-differentiation through activation of cell-intrinsic transcriptional programs. Extrinsic signals from the microenvironment superimpose on these programs to adapt Schwann cell function to a specific repair requirement of their surrounding tissue.[2] In this case, the context is determined by the melanoma cells that adapt the program to its own needs.

### Invasiveness

What is the Schwann cell, where does it come from and how does it change the tumor microenvironment to aid melanoma invasion?

## INFLUENCE OF THE PERIPHERAL NERVOUS SYSTEM ON CANCER

The peripheral nervous system is an important element in the skin microenvironment. Until recently interaction between solid malignancies and the peripheral nervous system involved the presence of nerve fibers within tumor parenchyma causing neuropathic pain and encouraging spread of tumor by means of perineural infiltration. Primary melanoma is not normally associated with pain and perineural infiltration is not a significant mode of spread. However, it is now becoming apparent that the peripheral nervous system is an important factor in melanoma growth. It has also been implicated in progression of basal cell carcinoma, gastric, and prostate cancer.[3]

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The Schwann cell is the most numerous glial cell present in the peripheral nervous system and involvement of the neuroglia is being increasingly appreciated as playing a part in melanoma progression. Neuroglia are intimately associated with nerve fibers providing trophic support and a signaling scaffold for the axon as well as playing a substantial role in maintaining homeostasis in the peripheral nervous system.[4,5]

## THE NEURAL CREST-DERIVED SCHWANN CELL LINEAGE

Cells of the neural crest give rise to a number of sub-lineages, one of which is the Schwann cell, the predominant glial cell of peripheral nerves. Neural crest cells transform into adult Schwann cells through two intermediate stages, the Schwann cell precursor (SCP) in the early embryonic stage, and immature Schwann cells appearing in late embryonic and perinatal stage nerves.

The SCP forms when it enters into an intimate relationship with an axon. SCPs retain developmental multipotency and contribute to other crest-derived lineages during development, including melanocytes and some neurons. [Figure 1] Finally, in post-natal nerves immature cells are induced by axon-associated signals to form myelinating and non-myelinating (Remark) Schwann cells of adult nerves.

A fourth phenotypic transformation is potentially triggered by nerve injury in which adult cells are changed into repair Schwann cells, major changes in gene expression enabling orchestration of nerve regeneration.

## NERVE INJURY REPAIR

The adult peripheral nervous system retains significant regenerative potential, even with complete transection. Unlike central nervous system axons, damaged peripheral nerve axons can regrow.[6] The process dependent on plasticity of the peripheral nervous system glia.[7] After peripheral nerve injury, repair occurs through a set of steps orchestrated by the Schwann cell. Schwann cells immediately loose connection with the nerve distal to the injury, which regresses through Wallerian degeneration [Figure 2].

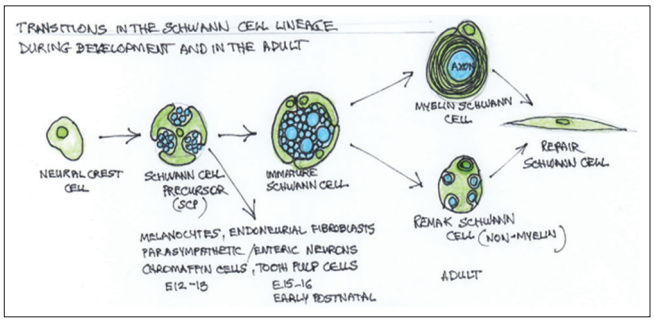


**Figure 1:** Transitions in the Schwann cell lineage during development and in the adult (Adapted from Jessen *et al.*, 2015)

Within minutes of axonal injury $Ca^{2+}$ enters the nerve initiating axonal breakdown. DAMPS stimulate de-differentiation of Schwann cells, through upregulation of Jun and erbB2. Through TLR signaling de-differentiated Schwann cells upregulate pro-inflammatory molecules (green box) increased TNFα and IL-1β upregulate MMP-9 and CCL2 and within hours fibroblasts upregulate IL-6 and GM-CSF. Repair Schwann cells (rSC) respond to IL-6 from fibroblasts further upregulating CCL2. Neutrophils infiltrate briefly within day 1. By 3 days post-injury macrophages have accumulated, phagocytosing the myelin debris, resulting from ongoing degenerative processes. There are two sites of action of macrophages in response to nerve injury, the distal nerve and around the injured neuronal bodies. They also contribute to inflammation by production of TNFα and IL-1β. Phagocytosis is augmented by resident macrophages. M1 markers are present early but become M2 markers beyond day 3, and remain elevated for 14 days. Once myelin degeneration is complete there is an upregulation of anti-inflammatory IL-10 leading to downregulation of pro-inflammatory cytokines. Removal of debris, which is inhibitory to regenerating axons, is a prerequisite for successful regeneration. CCL2, chemokine C-C motif ligand 2; DRG, dorsal root ganglion; DAMPS, danger-associated molecular patterns; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; Jun, gene for transcription factor AP-1; LIF, leukemia inhibitory factor; MMP-9, matrix mellatoproteinase-9; TLF, toll-like receptor; and TNFα, tumor necrosis factor-α (adapted from Defrancoesco-Lisowitz 2015).

This stimulates major changes in gene expression in the Schwann cell leading to transdifferentiation of the Schwann cell into a rSC. There is upregulation of markers of immaturity (Cd2, Egr1, c-Jun, and Sox2) and downregulation terminal differentiation genes (Sox10, Egr2, and Oct6). The Schwann cells then switch off myelination programs, acquire a new phenotype and together with fibroblasts secrete cytokines that promote infiltration of immune cells, coordinating, and supporting nerve repair. There is;

- Secretion of neurotrophic factors promoting axonal survival
- Clearance of myelin and expression of axonal guidance and adhesive clues to generate a favorable environment for axonal regrowth
- An inflammatory response promoting wound healing
- Proliferation to replace lost cells[6]
- Formation of a nerve bridge with provision of connective tissue across the gap involving extracellular matrix remodeling.

Schwann cell dedifferentiation to a progenitor-like cell underlies the ability of peripheral nerve regeneration. Transcription factors, epigenetic changes, and signaling pathways have been uncovered

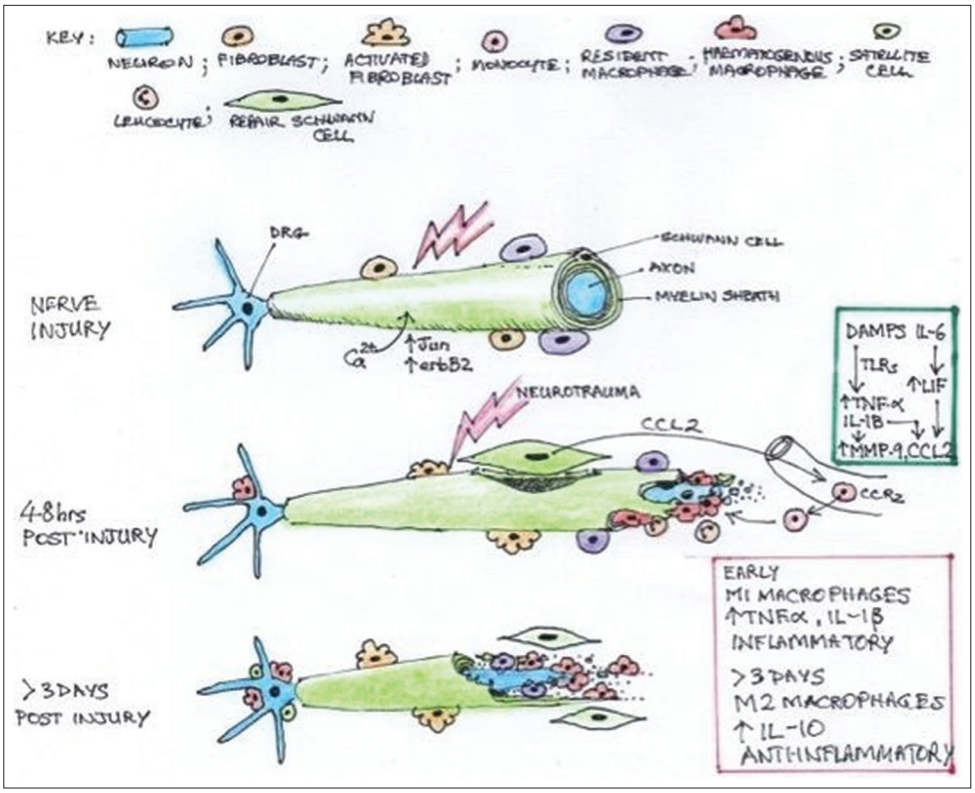


**Figure 2:** Cells and molecules involved in Wallerian degeneration

that control Schwann cell programming.[7] Work by Clements *et al*. was able to identify and isolate different populations from within the neural wound. Comparison between cells in the bridge, the area of neuronal loss after Wallerian degeneration, the distal nerve stump, and intact nerve showed different genetic and transcriptional profiles resulting in differential acquisition of mesenchymal traits. Bridge Schwann cells were identified as a distinct population with increased mesenchymal activity by localized increase in transforming growth factor β (TGFβ) signaling promoting Schwann cell invasion into the wound.[2] The TGFβ signaling was augmented by cross talk with Eph signaling to drive migration of Schwann cells across the wound. Wound fibroblasts provided the ligand for EphB2 receptors on Schwann cells which not only drove migration but also sorted Schwann cell into cords. N-cadherin being relocated to cell to cell contacts in a Sox2-dependent manner.[8] Clements *et al*. concluded that the wound microenvironment is the key determinant of Schwann cell identity.[2]

The rSCs can also disseminate from injured nerves into granulation tissue and support non-neuronal tissue repair.

## MACROPHAGE PHENOTYPE IN THE DISTAL NERVE SEGMENT

Changes in the macrophage phenotype occur in response to the cytokine environment, changing the activation state of the macrophages. M1 macrophages, present early but gone by day 3–4, are anti-inflammatory and cytotoxic. CCL2 signaling and secretion of IL-6 by macrophages and fibroblasts polarize macrophages toward a M2 phenotype that participates in tissue repair and wound healing. The DRG contains M2 macrophages. The M2 macrophages secrete IL-10 which downregulates both pro-inflammatory cytokines and macrophage MM-9, reducing macrophage infiltration. IL-10 secretion begins day 4, peaks day 7 and remains elevated till day 14, bringing Wallerian degeneration to an end, after clearance of degenerated myelin is complete.

## MELANOMA/PNS INTERACTION

As melanoma invades into the dermis the cells encounter a highly innervated tissue comprising of a dense network of myelinated and non-myelinated sensory and autonomic fibers of the peripheral nervous system. Using antibodies against axonal and Schwann cell markers, Shurin *et al*. found a reduction of neuronal fibers and Schwann cells throughout a majority of the melanoma tumor mass. However, nerves and Schwann cells were detected adjacent to the tumor. In contrast to melanoma, naevi demonstrated intact innervation. Immunostaining for GAP43 and $p75^{NTR}$, neuroplasticity markers of regenerating neurons and activated Schwann cells, respectively, was more abundant in adjacent tissue. Their conclusion was that with melanoma growth, intradermal nerve fibers are destroyed or displaced with induction of

nerve injury response and activation of denervated Schwann cells at the tumor edge.[3] Nerve injury accelerates the growth of melanoma resulting from modulation of the local microenvironment by activated rSCs. Promotion of epithelial to mesenchymal transition (EMT)-transcription factors activates tumor cells as well as the Schwann cell, increasing mobility, and transmigration.

## SCHWANN CELLS ARE IMMUNE COMPETENT CELLS

As well as changes in gene and transcription factor expression, there is also immune modulation. rSCs show an increased expression of a range of chemo-attractive agents (interleukins and TGFα) targeting immune cells, particularly myeloid regulatory cells, to control immunosuppressive potential of the tumor immunoenvironment.[9] Martyn *et al*. found that melanoma-treated Schwann cells, but not control Schwann cells, enhanced myeloid-derived suppressor cell's ability to suppress T cell proliferation *in vitro*. They also determined that the mechanism was related to an increased expression of myelin-associated glycoprotein (MAG), an inhibitor of axonal growth selectively localized in Schwann cells.[9]

## MELANOMA CELLS REPROGRAM SCHWANN CELLS

Recent studies have shown that melanoma-activated Schwann cells are functionally similar to nerve trauma-associated rSC. Both types supporting melanoma tumor growth *in vivo*.[7] The maintenance of plasticity in Schwann cells with the intention to assist nerve repair is utilized by melanoma cells to help create conditions favorable for tumor growth and progression.

Unlike other solid tumors, melanoma invasion of the dermis may destroy or displace nerves. This may explain the finding that significantly more neural tissue-related genes, particularly those upregulated after nerve injury, are expressed in tissue adjacent to the melanoma, while neurodegenerative processes are occurring within the tumor. Gene expression by nerves and Schwann cells at the tumor edge phenotypically mimicking responses to neurotrauma. Studies have shown that most intra-tumoral nerve or Schwann cell markers are strongly expressed by melanoma cells.[3] Chronic cancer-induced reprogramming of the Schwann cells in the tumor milieu is characterized by a non-resolving neurodegenerative process unlike normal tissue regeneration, being reminiscent of the concept of a non-healing wound. These melanoma-induced transactivated Schwann cells are functionally similar to Wallerian degeneration-associated rSC, with enhanced mobility, ECM reorganization, and macrophage attraction. This creates a paradigm were melanoma cells reprogram Schwann cells, initiating nerve injury response in surrounding tissues. Having a common neural crest progenitor, they share signaling axes with the peripheral nervous system including response to neurotrophic factors – nerve growth factor, ephrins, semaphorins, and neuregulins (NRGs). This results in accelerated tumor growth *in vivo* due to modulation of the local microenvironment by activated Schwann cells. In relation to NRG signaling, Schwann cells secrete NRG-1 and express epidermal growth factor-like receptors (ErbB2 and ErbB3) in response to axon damage, suggesting autocrine interaction. Autocrine regulation of cell proliferation and differentiation has been reported in other tissues and carcinomas. This autocrine activation of the ErbB2 and ErbB3 receptor complex (ErbB2 and 3 dimerize to produce a high affinity receptor) by NRG-1 has also been observed in non-small cell lung cancer, accelerating tumor growth and affecting prognosis.[10]

Dorsal root ganglia neurons, located within the melanoma-harboring dermatome, also express markers of nerve injury. Considering that axons and glia exist in a constant state of interaction, it is therefore likely that both axons and glia participate in the nerve repair response triggered by melanoma. Dorsal root ganglia may further promote tumor growth *in vivo*.[11]

## EMT AND STEMNESS

Plasticity is the ability of cells to reversibly change their phenotype and EMT is an example. Epithelial cells acquire a mesenchymal-like phenotype increasing migratory and invasive properties, naturally occurring in embryogenesis and cancer. The reverse process, MET, allows cells to settle and differentiate in different tissues and may be necessary for metastatic colonization. The EMT program confers stem cell-like properties on epithelial cells and in the context of cancer, give rise to tumor initiating cells.[11]

EMT is regulated by signaling pathways, particularly TGFβ, normally considered a tumor suppressor in epithelial cells, inhibiting growth and inducing apoptosis, but in tumors overcoming suppressor effects and responding to EMT with proliferation and survival. TGF-β1 overexpression correlates with tumor progression, metastases, and poor prognosis. These pathways are particularly susceptible to gain-of-function mutations and constitutive signal activation. EMT correlates with the appearance of a less differentiated phenotype, reminiscent of progenitor-like cells.[12]

## EMT-GRADIENT MODEL

EMT programs may present different threshold levels that couple or uncouple EMT from stemness. Initial activation allows acquisition of both migratory and stem-like features.

However, the fully committed mesenchymal phenotype may represent an alternative differentiation program where

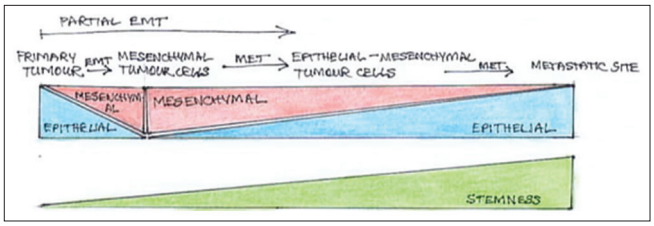


**Figure 3:** Epithelial to mesenchymal transition (EMT)-stemness interplay. Activation of the EMT program may present different threshold levels that couple/uncouple EMT from stemness (Adapted from Fabregat 2016)

stem cell-like features are lost. This may be controlled by microenvironmental factors, more mesenchymal during invasion and more epithelial-stem cell during colonization. The partial mesenchymal state, found in different carcinomas, controlled by multiple EMT programs with different usage of EMT-transcription factors, epigenetic, and metabolic programming and paracrine and autocrine signals, Figure 3.

## CONCLUSION

The establishment of the neural crest has been a huge step forward in the evolutionary development of the vertebrate species. It has allowed a progression from sessile filter feeder to mobile, aggressive predator by elaboration of a mechanism of adaptation to environmental variability. There has been the rolling out of a system of sensors at the periphery with communication channels back to the central system. There is a level of plasticity built in to the system to allow adaptation to changing environmental conditions. This means that cells of neural crest origin have common molecular, genetic, transcriptional, and signaling processes. There is a close relationship between the melanocytes, neurons, and glia. The melanoma cell is changed through a series of mutational events but is still part of this neural crest lineage and this allows it to manipulate peripheral systems of sensing and signaling to its own ends and explains some aspects of its ability to invade and metastasize. The final result is that melanoma is an independent, adaptable tissue that has proved difficult to control.

## FUTURE DIRECTIONS

High plasticity and abundance of neuroglia, and particularly the Schwann cell, warrants further laboratory and clinical investigation. There is the potential to control cancer pain which involves Schwann cell activity in the tumor microenvironment. Furthermore, the potential to target conditions such as Charcot-Marie-Tooth disease, Guillain-Barré syndrome, Neurofibromatosis, and Leprosy. Hopefully, even progress with the Tasmanian devil facial tumor disease is pushing this iconic native Australian marsupial to the point of extinction.

A better understanding of the influence of melanoma cell mass on neuroimmune circuitry in the microenvironment may open the way for alternative therapeutic approaches.

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