New insights into the neurofibroma tumor cells of origin

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Abstract

Neurofibromatosis type I (NF1) is a debilitating inherited tumor syndrome affecting around 1 in 3000 people. Patients present with a variety of tumors caused by biallelic loss of the tumor suppressor neurofibromin (NF1), a negative regulator of Ras signaling. While the mechanism of tumor formation is similar in the majority of NF1 cases, the clinical spectrum of tumors can vary depending on spatiotemporal loss of heterozygosity of *NF1* in cells derived from the neural crest during development. The hallmark lesions that give NF1 its namesake are neurofibromas, which are benign Schwann cell tumors composed of nervous and fibrous tissue. Neurofibromas can be found in the skin (cutaneous neurofibroma) or deeper in body near nerve plexuses (plexiform neurofibroma). While neurofibromas have been known to be Schwann cell tumors for many years, the exact timing and initiating cell has remained elusive. This has led to difficulties in developing animal models and successful therapies for NF1. A culmination of recent genetic studies has finally begun to shed light on the detailed cellular origins of neurofibromatosis. In this review, we will examine the hunt for neurofibroma tumor cells of origin through a historical lens, detailing the genetic systems used to delineate the source of plexiform and cutaneous neurofibromas. Through these novel findings, we can better understand the cellular, temporal, and developmental context during tumor initiation. By leveraging this data, we hope to uncover new therapeutic targets and mechanisms to treat NF1 patients.

Key Points

1 The spatiotemporal loss of NF1 during development leads to different types of neurofibroma formation.

2 The cells of origin for cutaneous and plexiform neurofibroma originate from a common stage in Schwann cell development.

3 Pinpointing the tumor cell of origin enables accurate modeling of neurofibromagenesis to unravel its fundamental biology and targeted therapy.

Neurofibromatosis Type 1

Neurofibromatosis Type I (NF1) is one of the most common tumor predisposition syndromes, which is inherited in an autosomal dominant fashion affecting 1 in 3000 live births.[1] Patients with NF1 are born with mutations in one allele of the tumor suppressor, Neurofibromin (also *NF1*), which is a negative regulator of Ras and MAPK signaling.[2,3] During development, loss of heterozygosity (LOH) of the other *NF1* locus results in the development of a spectrum of spatiotemporally distinct tumors and other clinical presentations depending on the cell-type affected. Some of these manifestations include neurofibromas, café au lait macules, optic gliomas, scoliosis, and learning disabilities.[4] Neurofibromas are the most

common presentation of NF1 and are benign tumors composed of Schwann-like cells, fibroblasts, a thick collagen matrix, mast cells, macrophages, nerves, and other cell types.[5] Over the past two to three decades, work by many labs have made significant progress in characterizing the biology and genetics of neurofibromas. However, the cell of origin and developmental context in which NF1 undergoes LOH has remained elusive. In this review, we will explore the importance of stem cells in NF1 tumorigenesis and the efforts by the neurofibromatosis scientific community to resolve the spatiotemporal loss of NF1 during development to identify neurofibroma tumor cells of origin.
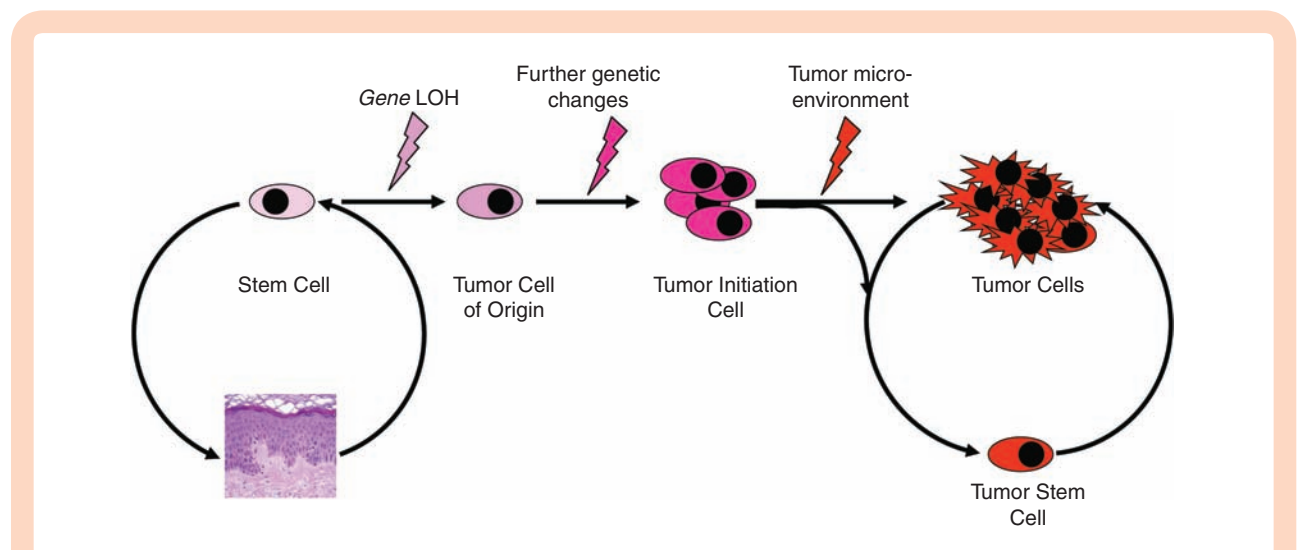
Stem Cell Models of Tumorigenesis: Tumor Cell of Origin vs Tumor Initiation Cell vs Tumor/Cancer Stem Cells

Throughout recent years, incredible efforts and resources have been dedicated toward understanding the roles that stem cells play in cancer pathogenesis. Their essential roles in development and regeneration are due to their unique characteristics of self-renewal and lineage plasticity.[6,7] Unsurprisingly, these characteristics are highly beneficial for the growth and initiation of tumors, and often hijacked during oncogenesis.[8,9] In the context of NF1, the wide array of tumor types and locations suggest that NF1 LOH occurs in an undifferentiated stem cell precursor during early development. The multi-faceted roles of stem cells in tumorigenesis and how they may relate to NF1, will be explored in the following sections.

Stem cells play a variety of important roles in cancer, however two of the most important roles are in tumor maintenance and initiation (Figure 1).[10] In classical models of cancer progression, all cells have equal ability to self-renew and generate tumorigenic cells. However, it is also possible that tumors may harbor stem cell populations that maintain and drive tumor growth (Figure 1). These cells are termed cancer stem cells (CSCs) and have received significant attention due to their implications for therapeutic treatment.[11,12] In this model, treatment with chemotherapy may kill the majority of cancer cells within a tumor, but not the CSCs.[13] The CSCs can, then, proliferate and differentiate to give rise to a new tumor, which clinically manifests as treatment relapse.[13] Recent work has shown that many genes expressed in stem cells during development become reactivated during cancer. Genes such as *Twist1* have been shown to play significant roles in regulating resistance to chemotherapy.[9,14,15] Additionally, the epigenetically naïve state of stem cells may also provide plasticity and resistance in the context of cancer.[16] In this regard, identification of therapies that target CSCs is required for successful cancer treatment.

While stem cells can play key roles in maintaining developed tumors, they can also play an important role in tumor initiation. Given their proliferative nature and abundance of euchromatin, stem cells are often hotspots for mutational acquisition.[16] This positions them as prime candidates for tumor cells of origin (Figure 1). Evidence in many systems have shown that the initial mutations can occur in stem cell populations and remain dormant.[10,17] Over time, these stem cells differentiate and acquire additional mutations leading to tumor formation.[17] These cells are often termed tumor initiation cells (Figure 1), representing the mutated population of cells undergoing the initial expansion. The importance of multipotent tumor cells of origin may explain why inherited mutations in key tumor suppressors or oncogenes often present as tumor syndromes,

Figure 1. The stem cell model of tumorigenesis. The stem cell model of tumorigenesis has three separate but related components. The first component concerns whether tumors arise from tissue adult stem cells or their immediate progenitors. These tumor cells of origin are cells that acquire first genetic mutation or loss of heterozygosity (LOH). The second component concerns tumor initiation cells which are cells that undergo the initial expansion secondary to further genetic changes or external signals from the microenvironment. It is entirely possible that in some instances, the tumor cells of origin and the tumor initiation cells are the same cell type. The third component of the stem cell model of tumorigenesis is that tumors are sustained and driven by a small population of cells with stem cell properties, termed cancer, or tumor stem cells.

Figure 2. Different types of neurofibroma. Cutaneous neurofibromas appear at puberty in the skin and they are exclusively in the dermis (A). Plexiform neurofibromas are congenital and progressively enlarge throughout life. They are often non-circumscribed, involving multiple peripheral nerve bundles (B and C) or paraspinal nerves near the dorsal root ganglia (D, arrows).

such as NF1, that can affect multiple distinct tissues and locations.[18–21] It has been historically difficult to identify tumor cells of origin given their low abundance and stochastic formation[10]; however recent work using lineage-tracing has been incredibly beneficial in narrowing down this population in many cancer contexts, including the developmental origin of different tumor types in NF1.

Types of Neurofibroma

There are two major types of neurofibromas in NF1: cutaneous and plexiform (Figure 2). Cutaneous (also called dermal) neurofibromas (cNF) are the most common manifestation of NF1 and typically arise during puberty. They are found in nearly 100% of NF1 patients and originate from Schwann cell lineage in the dermis. They can present as large plaques of hyperpigmentation such as in diffuse cNF or as multiple individual polyp-like structures such as in discrete or nodular cNF.[22] The number of cNF can vary significantly between patients ranging from just a few hundreds to thousands. Despite their benign nature, cNF can cause significant morbidity and psychosocial distress due to their severe disfigurement.

Approximately 30% of NF1 patients will also harbor plexiform neurofibromas (pNF), which are pathognomonic for the disease.[23] These tumors are thought to be developmental in origin and can be present at birth. pNF arise from glial cells of internal nerve plexuses and can be found surrounding spinal nerves deep within the body.[22] While pNF are also benign tumors, they can pose two major threats. As they grow larger, pNF can lead to impingement and compression of important nerves and nerve roots. This mass effect can lead to significant limb paralysis and loss of sensation. Second, tumor cells can acquire further mutations in key genes such as p53 that may result in transformation of the pNF into a malignant peripheral nerve sheath tumor (MPNST).[24] This aggressive cancer is the most common cause of death in NF1 patients and each individual patient with pNF has an approximately 10% lifetime risk to develop MPNST.[25,26]

While cNF and pNF are both tumors of Schwann cell origin, the timing, location, and prevalence of tumor formation differs. pNF is present at birth and grow larger with age while cNF typically begins developing during adolescence and puberty.[22] Additionally, cNF have been known to grow in size and number during pregnancy, suggesting a hormonal component to their pathogenesis.[27] pNF are also found deeper in the body, while cNF are contained exclusively within the dermis. Lastly, nearly all patients with NF1 will present with cNF, but only 30% of patients will present with pNF.[23] These differences suggested that cNF and pNF may have distinct cellular origins. By defining the cells of origin for cNF and pNF, unique vulnerabilities can be uncovered that enable the development of targeted therapy for these tumors.

Neurofibroma Cell of Origin

The term neurofibroma was initially coined by Friederich von Recklinghausen in 1882.[28] He noticed the presence of both neuronal and fibrotic tissue within the tumor. Over the next century, extensive histological analysis identified abnormal proliferation of Schwann cells that led scientists to hypothesize a Schwann cell origin of neurofibromas.[29,30] Subsequently, it was shown that NF1 tumors harbored *NF1* LOH and that neurofibroma-derived Schwann cells but not fibroblasts showed invasive properties.[31,32] This hypothesis was further cemented when Schwann cells and fibroblasts obtained from an NF1 patient tumor were genotyped for the mutant NF1 allele.[33] They observed that Schwann cells but not fibroblasts had LOH within the NF1 locus.[33] However, it was not until 2002 that Schwann cells were definitively shown to be the lineage of origin for neurofibromas. Armed with the knowledge that Krox20 was vital for Schwann cell differentiation and myelination,[34] the Parada lab used a Krox20-Cre transgene in order to delete NF1 specifically from the Schwann cell lineage in mice.[35] They found that loss of NF1 from the Schwann cell lineage in a NF1 heterozygous background was capable of recapitulating human pNF.[35] This finding also supported the long-suspected idea that NF1

heterozygosity within fibroblasts, mast cells, and other cells of the microenvironment plays a significant role in NF1 pathogenesis.[36] Despite the success of using Krox20-Cre to generate a plexiform neurofibroma, a key question remained unanswered. If the Krox20-expressing cell is the true cell of origin for neurofibromas, then why do mice only develop pNF while human patients almost all develop cNF? This major discrepancy between the mouse and human sparked a rapid scramble over the next 1.5 decades to further delineate and resolve the exact cellular origin of neurofibromas.

In order to address the origins of neurofibroma, one must first understand the developmental origin of Schwann cells. Around embryonic days E12-13 in the mouse, a subpopulation of migrating neural crest stem cells (NCSCs) differentiates and gives rise to the Schwann cell precursor (SCP).[37] SCP are intimately associated with axons and retain some level of multipotency with the ability to generate fibroblasts and enteric neurons.[38–40] They are typically found only in association with embryonic nerves and express a variety of markers including GAP43, SOX10, BLBP, P75, PLP, and DHH.[37] Later during development and around the perinatal period, SCP undergoes differentiation into immature Schwann cells (iSch).[37] Here, they lose their survival dependence on axon secreted NRG1 and begin upregulating the Schwann cell markers S100 and GFAP.[37,41–43] Upon further signals from the microenvironment and a 1:1 association with axons, iSch can be stimulated to differentiate into mature myelinating Schwann cells.[37,44] These cells will, then, insulate axons of the peripheral nervous system to enable rapid electrical conduction. In the absence of proper cues, iSch will differentiate into a non-myelinating form of mature Schwann cells that leads to formation of Remak bundles.[37,44]

In addition to SCP, a subpopulation of migrating NCSCs can also differentiate into Boundary cap cells.[37,45–47] Boundary cap cells are transiently located at the entry and exit zone of nerve roots and they also give rise to Schwann cells, however they are more intimately associated with spinal nerve roots and can be found in the dorsal root ganglion.[45] These cells express Krox20 and play an important role in myelinating the dorsal and ventral nerve roots and their more distal projections.[37,45] A subpopulation within the Boundary cap cells was recently found to express Prss56 and lineage tracing experiments showed that they give rise to Schwann cells of ventral and dorsal nerve roots, dorsal root ganglia, dermis, and hypodermis[48] suggesting that the Boundary cap cell lineage might be one of the sources for embryonic neurofibroma tumor cells of origin.

There has been increasing evidence of Schwann cell lineage plasticity during development and differentiation.[37,49] This is particularly important in the context of neurofibroma, given the multiple cellular lineages that cannot only be affected by loss of NF1, but also play a role in neurofibroma formation. It has been hypothesized that upon nerve injury Schwann cells can de-differentiate to form an intermediate repair Schwann cell that stimulates the regeneration of damaged peripheral nerves.[50–53] These cells can, then, differentiate back into myelinating and non-myelinating Schwann cells once axons have been repaired.[37] Additionally, there is evidence suggesting that Schwann cells can give rise to melanocytes after injury, and that SCPs can differentiate into

the fibroblast lineage.[38,54] Given that café au lait macules (a phenotype of NF1 loss in melanocytes) are a common presentation of NF1 patients and fibroblasts are a key component of neurofibroma formation, it's possible that NF1 LOH occurs within a multipotent precursor of the neural crest and Schwann cell lineage. The timing of NF1 LOH may play a role given the wide spectrum of phenotypes present in NF1 patients. At its core, a significant number of these phenotypes arise in tissues and cells originating from the neural crest. The exact multipotent stem cell from which NF1 is lost likely dictates the subsequent phenotype.

The hypothesis that stem cells may serve as the cell of origin for mutational acquisition is becoming increasingly evident, particularly in the context of developmental tumors. Gliomas are thought to originate from mutations acquired in neural stem cells while a wide array of primary liver cancers, including hepatocellular carcinoma and intrahepatic cholangiocarcinoma, are hypothesized to arise from mutated hepatic progenitors cells.[55,56] The basis of this hypothesis revolves around the idea that stem cells during development can acquire key mutations leading to tumor initiation.[57] Over time, acquisition of additional mutations and exposure to a permissive microenvironment results in tumor progression and later transformation.[58] In the case of NF1, this model offers an appealing explanation for the spectrum of phenotypes that are observed in patients. Perhaps an unidentified SCP or NCSC undergoes LOH during development. This precursor could, then, differentiate into multiple lineages including Schwann cells of the spinal roots and the dermis, melanocytes, astrocytes, and other cell lineages originating from the neural crest. Over time, environmental changes or additional mutations can lead to tumor initiation and expansion. If these additional changes occur in Schwann cells of the spinal nerves and deeper nerve plexuses, then pNF forms. If they occur during adolescence in Schwann cell lineages of the dermal nerve endings, then cNF forms. This two-step model of tumorigenesis may explain the spatiotemporal heterogeneity of NF1 presentations. Another consequence of this two-step model of tumorigenesis hypothesis is the idea that neurofibromas can arise from multiple cells of origin. In this scenario, NF1 LOH is only required for priming and initiation of neurofibromas. This suggests that progression of the individual tumors can occur in Schwann cell lineages that are spatiotemporally distinct. This is supported by genetic mouse models which form either pNF or cNF, but never both. Additionally, the adult dermis contains neural crest derived precursors that are capable of developing into neurofibromas upon loss of NF1 and the right environmental conditions.[59] Whether all neurofibromas originate from a common cell of origin, different cell of origin, or somewhere in between remains to be seen. However, recent work has begun to shed light on this topic.

Plexiform Neurofibroma Cell of Origin

The first genetic mouse model of NF1 was generated using a Krox20-cre-mediated deletion of NF1 in an NF1^{+/−} background.[35] These mice reproducibly developed pNF and validated the Schwann cell lineage as the origins of this form of neurofibroma.[35] However, Krox20 is expressed by

NCSCs, Schwann cells, and Schwann cell progenitors.[34,45] Therefore, the exact timing and cell of NF1 loss still remained unclear. In 2008, a series of papers in *Cancer Cell* from the Ratner, Morrison, and Zhu labs provided further refinement of pNF cell of origin. Joseph et al. showed that deletion of NF1 in NCSCs using Wnt1-Cre and Periostin-Cre resulted in transient hyperproliferation of NCSCs but no tumor formation.[60] However, loss of NF1 in SCPs using a P0A-cre resulted in pNF formation.[60,61] This was also independently validated by Zheng et al. using the same P0A-cre mouse line.[62] They also suggested that non-myelinating SCPs of the Remak bundles were the cell of origin for neurofibromas.[62] In parallel, Wu et al. adenovirally delivered Cre into cultured NF1[f/f] DRG cells from E12.5 embryos.[63] In contrast to earlier studies which found that loss of NF1 in mature Schwann cells failed to trigger proliferation,[64] Wu et al. found that loss of NF1 in SCPs led to enhanced colony formation.[63] Through lineage tracing, they identified desert hedgehog (DHH) as an embryonic marker for SCPs and boundary cap cells.[63] Deletion of NF1 using a Dhh-Cre resulted in robust pNF formation, narrowing down the pNF potential cell of origin to an embryonic SCP.[63]

A key caveat in these experiments was the lack of spatio-temporal control over NF1 deletion due to usage of constitutive Cre-recombinases. Given that many of these genes are also expressed in the adult mouse, a non-embryonic cell of origin could not be ruled out. To tackle this, Le et al. utilized a PLPCre-ERT2 in order to delete NF1 at specific time points during development.[65] They verified activity of the PLPCre-ERT2 through lineage tracing and found it to be active embryonically in peripheral nerve plexuses and postnatally in the sciatic nerves, DRG, and trigeminal ganglion.[65] Using the PLPCre-ERT, they were able to show that pNF can form when NF1 is lost at different time points but more robustly in the SCP and iSch stages.[65]

In a follow-up study, Chen et al. further explored the cell of origin and timing of para-spinal pNF, which are the majority of internal pNF and harbor a greater chance of malignant transformation.[66] Given that these neurofibromas are found in close proximity to DRGs, they hypothesized that they may arise from PLP^{+} cells residing within the DRG.[66] In order to test this, they harvested embryonic DRG/nerve root neurosphere cells (DNSCs) from E13.5 Nf1[f/f]; R26R-LacZ embryos and infected them with adenoviral Cre to delete *Nf1*.[66] Upon implantation near the sciatic nerve, *Nf1*-deficient DNSCs robustly gave rise to pNF within 4 months.[66] In order to isolate the specific subpopulation of DNSCs which harbor tumorigenic potential, Chen et al. employed lineage tracing using PLPCre-ERT and a YFP reporter to sort the DNSC population into PLP[+] and PLP[−] populations.[66] The implanted PLP[+] but not the PLP[−] population gave rise to pNF, suggesting that PLP marks the specific pNF cell of origin.[66] Given that Dhh- and Krox20-expressing populations also give rise to pNF, they further showed that Krox20^{+} and Dhh^{+} cells are contained within the PLP[+] population[35,63,66] Additionally, loss of *Nf1* in implanted Krox20^{−} and Dhh[−] DNSCs also gave rise to pNF with high prevalence.[66] These data suggest that PLP[+] DNSCs represents a broader population susceptible to pNF formation than both the Krox20 and Dhh populations. This is further supported by the finding that PLP^{+} cells appear in the embryonic DRG at least 1 day prior to Krox20^{+} and Dhh^{+} cells.[66] In order to characterize the identity of the PLP[+] population, Chen et al. performed immunofluorescent analysis for a variety of markers. They showed that PLP[+] DNSCs express markers of the neural crest such as Nestin, P75, and Sox10 as well as markers of embryonic Schwann cells including BLBP, GFAP, and GAP43.[66] However, PLP^{+} cells did not express the iSch and mature Schwann cell marker, S100β.[66] Furthermore, PLP^{+} cells could be detected in the embryonic nerve roots at E10.5 and acute loss of *Nf1* within these early precursors resulted in pNF formation.[66] These findings demonstrate that the para-spinal pNF cell of origin is a PLP[+] SCP originating from embryonic nerve roots.

Cutaneous Neurofibroma Cell of Origin

In contrast to the relative wealth of work exploring pNF cell of origin, there is relatively little knowledge about the origins of cNF. This is especially important considering nearly 100% of NF1 patients present with cNF, while pNF is detected in only 30%. Additionally, robust mouse models of pNF do not give rise to cNF.[35,60,62,63,65] The conflicting clinical and mouse data therefore suggests one of 3 possibilities: (a) NF1 disease progression in mouse and human are fundamentally distinct, (b) pNF and cNF originate from different cells of origin, or (c) there is an even earlier and unidentified population of SCP that can give rise to both cNF and pNF.

The first reported mouse model developing cNF was generated by Saito et al. using a transgenic CAMK2-Cre driving overexpression of a constitutively active Nras (NrasG12V).[67] CAMK2-Cre[Tg/+]; NrasG12V[Tg/+] mice exhibited epidermal hyperpigmentation throughout life and developed diffuse cNF starting at 3 months of age.[67] Despite the broad expression of CAMK2 in the neural crest, the authors did not observe development of pNF or other characteristic lesions of NF1 patients.[67] These data suggest that cNF may also arise from neural crest progenitors, however the use of a constitutive Cre-recombinase precludes ruling out CAMK2-expressing cells later in development or adulthood. This is especially important given that cNF typically develops after birth unlike pNF which develops embryonically. Furthermore, transgenic overexpression of NrasG12V may not be reflective of NF1 pathogenesis at a gene dosage and functional level. While NF1 is predominantly thought to function in the Ras-MAPK signaling pathway, NrasG12V overexpression bypasses any alternative pathways that NF1 may signal through. In 2008, Wu et al. reported the generation of a cNF mouse model using the same Dhh-Cre; Nf1[f/f] used to generate pNF.[63] However, the cNF observed in that paper was found outside of the dermis below the panniculus carnosus.[63]

Support for distinct pNF and cNF tumor initiation cells came in 2009 when Le et al. identified that skin-derived neural progenitors (SKPs) could give rise to cNF upon loss of NF1.[59] SKPs are heterogeneous population of cells that contain resident neural and glial progenitors of the dermis which harbor neural crest-like properties.[68–71] When isolated and cultured, SKPs form neurospheres and can differentiate to form Schwann cells, neurons, and adipocytes.[59] Direct application of tamoxifen to the skin of neonatal CMV-CreERT2; Nf1[f/−] mice resulted in cNF formation at the site of application.[59] In order to show that SKPs were the culprit behind

cNF formation, tamoxifen-treated SKPs isolated from CMV-CreERT2; Nf1[f/−] mice were implanted into the sciatic nerve and dermis.[59] SKPs that were implanted near the sciatic nerve gave rise to pNF, suggesting that SKPs harbor intrinsic potential to generate neurofibromas upon loss of Nf1 expression.[59] SKPs implanted in the dermis only gave rise to classic cNF in hormone-primed recipient mouse.[59] This data suggests that a certain cell population within SKPs or resident cells within the dermis may be the cell of origin for cNF, and adds further support to the notion that hormonal milieu contributes significantly to cNF development. However, a few unanswered questions remain. Since SKPs are heterogeneous populations of cells, which subpopulations within them are capable of forming cNF? More importantly, is there a common cell of origin that is capable of forming both cNF and pNF when Nf1 is deleted, which would accurately recapitulate the human presentations.

Linking the Cell of Origin for pNF and cNF into a Common Stage/Phase in Schwann Cell Development

A significant breakthrough in addressing the cells of origins for pNF and cNF came recently when two independent groups generated mouse models of NF1 that recapitulated both cNF and pNF development. Chen et al. selected Hoxb7-Cre due to its specific labeling of neural crest derivatives that form the glia of dorsal nerve roots and the DRG, and migrate out to skin nerve endings along peripheral nerves in the dermis, as well as its expression in a subpopulation within the skin neurosphere cells.[72,73] When Nf1 was deleted using Hoxb7-Cre, isolated SKPs could form neurofibromas when implanted to the sciatic nerves.[72] This data suggests that Hoxb7-Cre is active in the SKP population and may mark the progenitors that give rise to cNF. To definitively show this, they analyzed Hoxb7-Cre; Nf1[f/−] and Hoxb7-Cre; Nf1[f/f] mice (H7;Nf1mut) and found that 64% of mice developed cNF by 1 year of age.[72] These cNF were of the diffuse-type, presenting as large, hyper-pigmented regions of thicker dermis.[72] A significant portion of the tumors stained positive for S100B and Sirius Red, validating that they were indeed neurofibromas.[74] Strikingly, approximately half the mice also developed signs and symptoms of pNF by 5 months of age.[74] Approximately equal numbers of mice had cNF, pNF, or both.[74] These observations indicate that HOXB7 serves as a lineage marker to trace the developmental origin of both cNF and pNF neoplastic cells. These findings represent the first reported incidence of a genetic mouse model that forms both cNF and pNF, and therefore accurately depicts the clinical presentation of NF1 patients.

At the same time, work from the Topilko lab identified boundary cap cells as having the ability to generate both pNF and cNF.[75] Using a Cre-recombinase knocked into the Prss56 locus, Radomska et al. showed that Prss56-Cre labels a subpopulation of boundary caps cells which subsequently gives rise to Schwann cells of ventral and dorsal nerve roots, dorsal root ganglia, dermis, and hypodermis.[75] Prss56-Cre;Nf1[f/−] and Prss56-Cre;Nf1[f/f] mice (P56;Nf1mut) both developed pNF and diffuse cNF with high penetrance.[75] When compared to skin labeled by Krox20- and Dhh-cre, Prss56-Cre uniquely labeled the subepidermal glia.[75] This suggests that subepidermal glia derived from boundary cap cells can give rise to cNF, explaining why previous models of NF1 were only able to generate pNF. They also found that skin injury accelerated cNF formation, likely due to reactivation and potentially dedifferentiation of Schwann cells.[74]

The work from these two groups have finally reconciled years of apparent discrepancy between mouse and human presentations of NF1. It appears that Hoxb7- and Prss56-expressing boundary cap cells/SCPs, which originate from a subpopulation of migrating NCSCs, likely represent the neurofibroma lineage of origin. Furthermore, the individual cell or cells responsible for pNF are likely residing in the DRG and spinal nerves and roots, while those responsible for cNF are the dermal glia that migrated from the dorsal nerve roots. In this model, cNF and pNF have the same cell of origin, but different initiating cells (Figure 3). cNF and pNF both appear to derive from boundary cap cells or a subpopulation of SCPs or even an earlier stage,
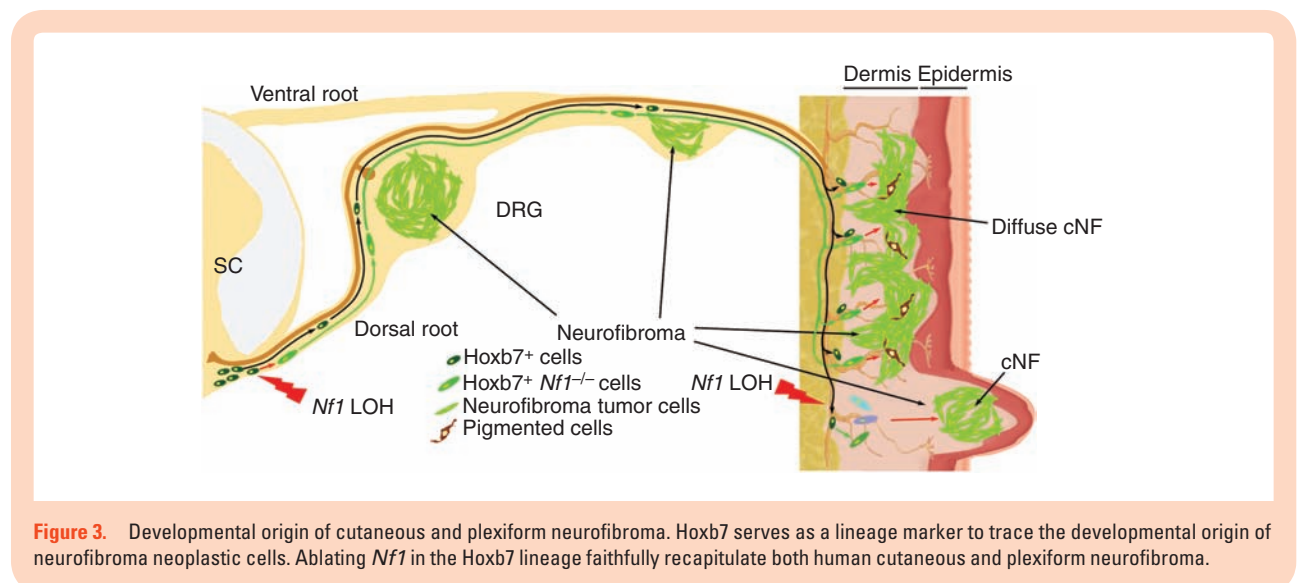
Figure 3. Developmental origin of cutaneous and plexiform neurofibroma. Hoxb7 serves as a lineage marker to trace the developmental origin of neurofibroma neoplastic cells. Ablating *Nf1* in the Hoxb7 lineage faithfully recapitulate both human cutaneous and plexiform neurofibroma.

however the spatiotemporal onset of neurofibroma formation differs. One could therefore argue that cNF and pNF originate from the same cell, a HoxB7- and Prss56-expressing boundary cap cell or SCP during embryonic development. However, spatiotemporal loss of NF1 function in the subsequent stages of this common cell of origin leads to different types of neurofibroma. Therefore, the current model also provides further support for the tumor stem cell and two-step tumorigenesis hypotheses described earlier. As was hypothesized, it appears that NF1 LOH first appears in the boundary cap cell/SCP population. These boundary cap cells represent the tumor cell of origin. Some of these cells will remain in proximal regions while others will migrate to the periphery to become subepidermal glia. Subsequently, the timing and location of tumor initiation determines the specific type of neurofibroma formed. This is also supported by data from Radomska et al showing the initial presence of micro-cNFs within the dermis, which develop over time into full cNF.[75] This finding suggests that neurofibroma progression is a step-wise process that likely depends on acquisition of further intrinsic or extrinsic signals from the environment. This also suggests that specific factors within the proximal PNS niche may accelerate neurofibroma formation, given that pNF typically occurs much earlier than cNF. In contrast, it is also likely that LOH of NF1 expression in the HoxB7- and Prss56-lineage cells in the skin later on in life, in addition to other microenvironmental cues (such as hormones, neurons, inflammation, injury, etc.), leads to cutaneous neurofibroma formation.

In spite of the significant leap in knowledge regarding neurofibroma cell of origin, several key questions remain unclear. Both the HoxB7- and Prss56-Cre models use a constitutive Cre-recombinase, which prevents controlled spatiotemporal deletion of Nf1. This may explain the discrepancy in the pattern of cNF and pNF acquisition between the mouse and human. In the HoxB7-Cre model, similar numbers of mice had pNF, cNF, or both, while humans typically always present with cNF. In these studies, a strong pNF phenotype typically necessitates euthanasia of mice, regardless of age. Therefore, it is possible that by the time the mouse is euthanized, cNF has not had time to fully develop. This technical caveat and the identification of micro-cNFs as precursor lesions warrants a closer look at previous NF1 mouse models that seemingly only give rise to pNF. In humans, loss of NF1 is spatiotemporally sporadic and therefore more time is given for cNF pathogenesis. Usage of an inducible Cre-ERT2 may enable NF1 LOH in a smaller population of cells such that mice live longer to develop cNF with 100% penetrance. Another discrepancy between mouse and human models of NF1 is that humans develop both discrete and diffuse cNF, while Hoxb7 and Prss56-Cre mice develop more diffuse cNF in the absence of further genetic alterations.[72,75] Usage of a constitutive Cre-recombinase likely targets the entire subepidermal glial population leading to diffuse cNF formation. A Cre-ERT2 may therefore overcome this problem as well by reducing the number of cells targeted and enabling targeting later in life. Such a tool will have immense benefit in further dissecting the susceptible stages of both pNF and cNF development.

Conclusion

In recent years, significant efforts have been dedicated toward identifying tumor cells of origin. The benefits of this knowledge are immense for our understanding of fundamental biology as well as targeted therapy. Pinpointing a tumor cell of origin enables the accurate modeling of disease initiation and progression. It also enables the identification of stepwise molecular drivers that promote human cancer progression. By understanding these steps, we can identify key targetable vulnerabilities within cancer cells. In the context of NF1, it will be of tremendous help to understand the subsequent events that lead from Nf1 LOH to neurofibroma development.

An additional benefit of identifying tumor cells of origin relates to the increasingly accepted role of epigenetics in cancer development and progression.[76–79] In the context of NF1, very little is known about the epigenetic changes that occur during tumor pathogenesis.[80] In contrast, many other cancers originating from developmental progenitors have extensive transcription factor binding and histone modification analysis.[81–86] These studies are incredibly useful in understanding how cellular epigenetic state may create permissive environments for tumor development and progression.[87] In this regard, identifying the NF1 cell of origin will enable us to study the chromatin environment and context of the original cell in which NF1 LOH occurs. This knowledge will serve as a useful base to put existing and future data into a proper context.

Lastly, the work and data presented in this review will enable the development of mouse models that recapitulate human NF1 progression and presentation. These models will serve as a platform for high-throughput screening of therapeutic compounds. Currently, very little therapy exists for neurofibroma in NF1 patients outside of surgical removal methods.[88] This gap in the current understanding of neurofibroma biology and clinical outcomes could be due to the lack of a robust pre-clinical model that accurately depicts NF1 pathogenesis. Further resolution of the neurofibroma cell of origin will be of utmost importance to fill this knowledge gap in reducing the morbidity and mortality for NF1 patients.

Keywords

cutaneous neurofibroma | neurofibroma cell of origin | neurofibromatosis type 1 | plexiform neurofibroma | tumor cell of origin

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