Cancer Microenvironment (2012) 5:307–310
DOI 10.1007/s12307-012-0115-x

REVIEW ARTICLE

The Plexiform Neurofibroma Microenvironment

Feng-Chun Yang · Karl Staser · D. Wade Clapp

Received: 26 April 2012 / Accepted: 10 July 2012 / Published online: 24 July 2012

Abstract Dynamic interactions between tumorigenic cells and surrounding cells, including immunomodulatory hematopoietic cells, can dictate tumor initiation, progression, and transformation. Hematopoietic-stromal interactions underpin the plexiform neurofibroma, a debilitating tumor arising in individuals afflicted with Neurofibromatosis type 1 (NF1), a common genetic disorder resulting from mutations in the *NF1* tumor suppressor gene. At the tissue level, plexiform neurofibromas demonstrate a complex microenvironment composed of Schwann cells, fibroblasts, perineural cells, mast cells, secreted collagen, and blood vessels. At the cellular level, specific interactions between these cells engender tumor initiation and progression. In this microenvironment hypothesis, tumorigenic Schwann cells secrete pathological concentrations of stem cell factor, which recruit c-kit expressing mast cells. In turn, activated mast cells release inflammatory effectors stimulating the tumorigenic Schwann cells and their supporting fibroblasts and blood vessels, thus promoting tumor expansion in a feed-forward loop. Bone marrow transplantation experiments in plexiform neurofibroma mouse models have shown that tumorigenesis requires *Nf1* haploinsufficiency in the hematopoietic compartment, suggesting that tumor microenvironments can depend on intricate interactions at both cellular and genetic levels. Overall, our continued understanding of critical tumor-stromal interactions will illuminate novel therapeutic targets, as shown by the first-ever successful medical treatment of a plexiform neurofibroma by targeted inhibition of the stem cell factor/c-kit axis.

Keywords NF1 · Tumor microenvironment · Mast cells · Neurofibromas

F.-C. Yang · K. Staser · D. W. Clapp
Herman B Wells Center for Pediatric Research,
Department of Pediatrics, Indiana University School of Medicine,
Indianapolis, IN, USA

F.-C. Yang
Department of Anatomy and Cell Biology,
Indiana University School of Medicine,
Indianapolis, IN, USA

K. Staser · D. W. Clapp
Department of Biochemistry,
Indiana University School of Medicine,
Indianapolis, IN, USA

D. W. Clapp
Department of Microbiology and Immunology,
Indiana University School of Medicine,
Indianapolis, IN, USA

F.-C. Yang (✉)
Herman B Wells Center for Pediatric Research,
Department of Anatomy and Cell Biology,
1044 West Walnut Street,
Indianapolis, IN 46202, USA
e-mail: fyang@iupui.edu

Also known as von Recklinghausen's disease, Neurofibromatosis type 1 (NF1) is a pandemic genetic disorder afflicting about 1 in 3,000 persons worldwide [1]. NF1 occurs as consequences of mutations of *NF1* tumor suppressor gene on the long arm of chromosome 17, a gene encoding neurofibromin [2]. Neurofibromin functions as a Ras GTPase activating protein (Ras-GAP), which accelerate the hydrolysis of active GTP-bound Ras to inactive GDP-bound Ras [3, 4]. *NF1* is highly conserved across species, especially in the functionally critical GAP-related domain [4, 5].

Although disease penetrance is complete, specific manifestations can vary widely from patient to patient. Patients are predisposed to both malignant and non-malignant conditions, such as cutaneous neurofibromas, plexiform neurofibromas, malignant peripheral nerve sheath tumors (MPNSTs), myeloid leukemia, vascular pathologies, and soft tissue malformation [6, 7]. Approximately 30 % of

individuals with NF1 suffer from plexiform neurofibromas, a tumor suspected to arise during gestation which can be an early source of disfigurement, disability, and lethality [8]. Intriguingly, plexiform neurofibromas demonstrate an intricate microenvironment composed of irregularly growing Schwann cells, large numbers of degranulating mast cells, perineural cells, collagen-secreting fibroblasts, and blood vessels.

Studies in other cancer models have demonstrated that tumorigenic cells and their inflammatory microenvironment affect tumor cell growth, transformation, and metastasis [9]. Although cancers frequently originate secondary to activation of oncogenes or alternatively as a loss of tumor suppressor genes in proliferating cells, it has become increasingly evident that a single mutagenic event is rarely sufficient for oncogenesis [10]. In a range of murine tumor models including breast, ovarian [11], pancreatic [12], and skin models [13], inflammatory cells play critical roles in maintaining the microenvironment that supports tumor genesis, progression, and transformation.

Microenvironments consist of various cell types, cytokines, growth factors, and components of the extracellular matrix. Importantly, the microenvironment, as a dynamic entity, differs in cellular and molecular specificity according to both tumor type and disease stage. For example, early cues from nascent tumor cells can recruit hematopoietic cells which secrete factors to promote local mitogenesis as well as the recruitment of additional immunomodulators. Further secretion of secreted cytokines and growth factors not only remodels the extracellular matrix and the vasculature, but also may provide the specific signals permitting cellular transformation and metastasis.

Mast cells are granular, tissue-resident immune effector cells derived from early myeloid progenitor cells in hematopoietic tissue [14]. Upon activation of the high-afinity IgE receptor (Fc RI) and/or the c-kit receptor tyrosine kinase, mast cells release inflammatory mediators including histamine, serotonin, proteoglycans, and leukotrienes [15]. In various disease models, mast cells can both positively and negatively regulate inflammation [16, 17]. In NF1-associated plexiform neurofibromas, inflammatory mast cells not only pervade tumor tissue and promote cellular growth [18–25], but they appear to be *required* for tumorigenesis. As support, hematopoietic ablation of the gene encoding the receptor tyrosine kinase c-kit (a key regulator of mast cell generation and bioactivity) prevents tumorigenesis in a plexiform neurofibroma murine model [25]. Furthermore, imatinib mesylate (Gleevec©), an inhibitor of c-kit and other receptor tyrosine kinases, successfully reduced a highly morbid plexiform neurofibroma in a pediatric patient with NF1 [25].

The presence of mast cells in tumor tissue of neurofibromas was initially reported by Greggio in 1911 [26]. Independent groups later reported the existence of mast cells within peripheral nerve sheaths, nerve sheath tumors, and NF1-associated neurofibromas [27–31]. Further histological analyses have shown significant increases of mast cell number in only the neurofibroma tissue, as compared to surrounding tissue [32]. Based on these observations and his own clinical observations, Vincent Riccardi hypothesized that mast cells might directly modulate neurofibroma tissue [33], although histamine-release inhibitors, while effective at symptom control, ultimately did not affect neurofibroma progression [34, 35].

Mouse models of plexiform neurofibroma formation have illuminated cellular and molecular interactions underpinning the tumor microenvironment while providing new insight into targeted therapeutic strategies. Zhu et al. created the first successful mouse model mimicking human neurofibroma genesis [18]. This mouse carries Cre recombinase placed under control of the *Krox20* promoter (*Krox20cre*), a promoter element preferentially expressed in a subset of Schwann cells. Surprisingly, *Krox20cre* mice crossed into $Nf1^{flox/flox}$ mice ($Krox20creNf1^{flox/flox}$), despite *Nf1*-deficient Schwann cells, are essentially healthy. Zhu et al. then showed that the presence of a constitutively null *Nf1* allele ($Krox20creNf1^{flox/-}$) successfully engenders tumorigenesis along the dorsal root ganglion. Histologic analyses demonstrated similarity between the murine tumors and human plexiform neurofibromas. Thus, plexiform neurofibroma formation in this model requires that a subset of Schwann cells are *Nf1* nullizygous while surrounding cells of the putative microenvironment, such as fibroblasts, endothelia, and mast cells, are *Nf1* heterozygous.

Bone marrow transplantation experiments in this mouse model demonstrated that neurofibroma formation hinges on *Nf1* haploinsufficient and c-kit-dependent bone marrow. To accomplish these experiments, Yang et al crossed $Nf1^{+/-}$ mice with mice carrying two distinct *W* mutations, a mutation which compromises c-kit receptor tyrosine kinase activity and inhibits mast cell production and bioactivity [36]. Bone marrow from wild-type, $Nf1^{+/-}$, $Nf1^{+/-};W^{41}/W^{41}$ or $Nf1^{+/-};W^{v}/W^{v}$ mice were transplanted into $Nf1^{flox/flox}$; *Krox20cre* mice. Recipients of either WT or *W* mutated bone marrow did not develop tumors, while recipients of *Nf1*+/– bone marrow formed tumors comparable to those found in $Krox20creNf1^{flox/-}$ mice. Conversely, transplantation of WT bone marrow into $Krox20creNf1^{flox/-}$ mice prevented tumor formation. Therefore, in this mouse model, the neurofibroma microenvironment genetically requires *Nf1* nullizygous Schwann cells and $Nf1^{+/-}$, c-kit-dependent bone marrow.

Various biochemical studies have helped delineate the hypothetical mechanisms by which $Nf1^{+/-}$ mast cells engender neurofibroma genesis. $Nf1^{-/-}$ Schwann cells secrete high levels of SCF, driving the generation and recruitment of mast cells [20, 37]. Specifically, media conditioned by

Nf1$^{-/-}$ Schwann cells attracted *Nf1*$^{+/-}$ mast cells at twice the rate of WT mast cells. These *Nf1*$^{+/-}$ mast cells, in turn, can secrete inflammatory cytokines and growth factors which promote the growth of Schwann cells, fibroblasts, and endothelial cells [20, 38–40]. Moreover, SCF-stimulated *Nf1*$^{+/-}$ mast cells, as compared to WT mast cells, proliferate more quickly, demonstrate enhanced survival, and secrete greater concentrations of inflammatory cytokines [38, 41–43]. In a subsequent study, SCF-stimulated *Nf1*$^{+/-}$ mast cells promoted fibroblast growth and collagen deposition [38], key cellular components of human neurofibromas [44].

These mechanistic insights propelled investigation into pharmacological inhibition of c-kit as a method to reduce and/or inhibit plexiform neurofibromas. Imatinib mesylate binds and inhibits the c-kit receptor tyrosine kinase, as well as c-abl, PDGF-β, TGF-β, and perhaps other RTKs [45]. Indeed, imatinib treatment of adult Krox20cre*Nf1*$^{flox/-}$ mice reduced neurofibroma volume and metabolism approximately 50 % as compared to placebo-treated controls. Nerve sheath samples from these mice showed restoration of morphology to Schwann cells and the absence of mast cells. Furthermore, imatinib treatment of a pediatric index patient with a debilitating neurofibroma reduced tumor volume 70 % while mitigating symptoms of airway and nerve compression [25].

Collectively, these genetic and pharmacological data argue that *Nf1*-deficient Schwann cells recruit and activate marrow-derived mast cells, which critically modulate tumor genesis and growth. While data from certain mouse models suggest that tumor formation requires *Nf1*$^{+/-}$ mast cells, other models using developmentally earlier and more widespread glial cell deletions have not shown this requirement for *Nf1*$^{+/-}$ mast cells [46]. Regardless, all mouse models to date have shown the pervasive presence of degranulating mast cells within the tumor tissue.

The tumor microenvironment is complex and dynamic, and varies depending on the type of tumor or cancer. Here, we have summarized historical and current evidence regarding the inflammatory microenvironment of the plexiform neurofibroma. SCF-recruited mast cells secrete multiple key growth factors that modulate the behavior of the neurofibroma stroma, and these mechanistic insights have led to a promising therapeutic strategy. Further studies of the cellular/molecular interactions between tumor, hematopoietic, and stromal cells in the neurofibroma microenvironment will undoubtedly unravel more clues in the search for targeted therapeutic interventions.

References

1. Wallace MR et al (1990) Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. Science 249(4965):181–186
2. Morris M, Fox WS (1908) Von Recklinghausen's Disease. Proc R Soc Med 1(Dermatol Sect):16–17
3. Martin GA et al (1990) The GAP-related domain of the neurofibromatosis type 1 gene product interacts with ras p21. Cell 63(4):843–849
4. Ballester R et al (1990) The NF1 locus encodes a protein functionally related to mammalian GAP and yeast IRA proteins. Cell 63(4):851–859
5. Hiatt KK et al (2001) Neurofibromin GTPase-activating protein-related domains restore normal growth in Nf1−/− cells. J Biol Chem 276(10):7240–7245
6. Bader JL (1986) Neurofibromatosis and cancer. Ann N Y Acad Sci 486:57–65
7. Friedman J et al (1999) Neurofibromatosis: phenotype, natural history, and pathogenesis, 3rd edn. The Johns Hopkins University Press, Baltimore
8. Korf BR (1999) Plexiform neurofibromas. Am J Med Genet 89 (1):31–37
9. Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420(6917):860–867
10. Coussens LM, Werb Z (2001) Inflammatory cells and cancer: think different! J Exp Med 193(6):F23–F26
11. Kacinski BM (1995) CSF-1 and its receptor in ovarian, endometrial and breast cancer. Ann Med 27(1):79–85
12. Bergers G et al (2000) Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2(10):737–744
13. Coussens LM et al (1999) Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. Genes Dev 13:1382–1397
14. Franco CB et al (2010) Distinguishing mast cell and granulocyte differentiation at the single-cell level. Cell Stem Cell 6(4):361–368
15. Galli SJ, Maurer M, Lantz CS (1999) Mast cells as sentinels of innate immunity. Curr Opin Immunol 11(1):53–59
16. Theoharides TC, Conti P (2004) Mast cells: the Jekyll and Hyde of tumor growth. Trends Immunol 25(5):235–241
17. Metz M et al (2006) Mast cells can enhance resistance to snake and honeybee venoms. Science 313(5786):526–530
18. Zhu Y et al (2002) Neurofibromas in NF1: Schwann cell origin and role of tumor environment. Science 296(5569):920–922
19. Viskochil DH (2003) It takes two to tango: mast cell and Schwann cell interactions in neurofibromas. J Clin Invest 112(12):1791–1793
20. Yang FC et al (2003) Neurofibromin-deficient Schwann cells secrete a potent migratory stimulus for Nf1+/− mast cells. J Clin Invest 112(12):1851–1861
21. Hirota S et al (1993) Possible involvement of c-kit Receptor and its ligand in increase of mast cells in neurofibroma tissues. Arch Pathol Lab Med 117:996–999
22. Staser K, Yang FC, Clapp DW (2010) Mast cells and the neurofibroma microenvironment. Blood 116(2):157–164
23. Staser K, Yang FC, Clapp DW (2012) Pathogenesis of plexiform neurofibroma: tumor-stromal/hematopoietic interactions in tumor progression. Annu Rev Pathol 7:469–495
24. Staser K, Yang FC, Clapp DW (2010) Plexiform neurofibroma genesis: questions of Nf1 gene dose and hyperactive mast cells. Curr Opin Hematol 17(4):287–293
25. Yang FC et al (2008) Nf1-dependent tumors require a microenvironment containing Nf1+/−− and c-kit-dependent bone marrow. Cell 135(3):437–448
26. Greggio H (1911) Les cellules granuleuses (Mastzellen) dans les tissus normaux et dans certaines maladies chirurgicales. Arch Med Exp 23:323–375
27. Pineda A (1965) Mast cells–their presence and ultrastructural characteristics in peripheral nerve tumors. Arch Neurol 13(4):372–382
28. Gamble HJ, Goldby S (1961) Mast cells in peripheral nerve trunks. Nature 189:766–767
29. Olsson Y (1971) Mast cells in human peripheral nerve. Acta Neurol Scand 47(3):357–368

30. Isaacson P (1976) Mast cells in benign nerve sheath tumours. J Pathol 119(4):193–196
31. Baroni C (1964) On the relationship of mast cells to various soft tissue tumours. Br J Cancer 18:686–691
32. Nurnberger M, Moll I (1994) Semiquantitative aspects of mast cells in normal skin and in neurofibromas of neurofibromatosis types 1 and 5. Dermatology 188(4):296–299
33. Riccardi VM (1981) Cutaneous manifestation of neurofibromatosis: cellular interaction, pigmentation, and mast cells. Birth Defects Orig Artic Ser 17(2):129–145
34. Riccardi VM (1987) Mast-cell stabilization to decrease neurofibroma growth. Preliminary experience with ketotifen. Arch Dermatol 123(8):1011–1016
35. Riccardi VM (1993) A controlled multiphase trial of ketotifen to minimize neurofibroma-associated pain and itching. Arch Dermatol 129(5):577–581
36. Galli SJ, Tsai M, Wershil BK (1993) The c-kit receptor, stem cell factor, and mast cells. What each is teaching us about the others. Am J Pathol 142(4):965–974
37. Chen S et al (2010) Nf1−/− Schwann cell-conditioned medium modulates mast cell degranulation by c-Kit-mediated hyperactivation of phosphatidylinositol 3-kinase. Am J Pathol 177(6):3125–3132
38. Yang FC et al (2006) Nf1+/− mast cells induce neurofibroma like phenotypes through secreted TGF-beta signaling. Hum Mol Genet 15(16):2421–2437
39. Li F et al (2006) Neurofibromin is a novel regulator of RAS-induced signals in primary vascular smooth muscle cells. Hum Mol Genet 15(11):1921–1930
40. Munchhof AM et al (2006) Neurofibroma-associated growth factors activate a distinct signaling network to alter the function of neurofibromin-deficient endothelial cells. Hum Mol Genet 15 (11):1858–1869
41. McDaniel AS et al (2008) Pak1 regulates multiple c-Kit mediated Ras-MAPK gain-in-function phenotypes in Nf1+/− mast cells. Blood 112(12):4646–4654
42. Ingram DA et al (2000) Genetic and biochemical evidence that haploinsufficiency of the Nf1 tumor suppressor gene modulates melanocyte and mast cell fates in vivo. J Exp Med 191(1):181–188
43. Khalaf WF et al (2007) K-ras is critical for modulating multiple c-kit-mediated cellular functions in wild-type and Nf1+/− mast cells. J Immunol 178(4):2527–2534
44. Jaakkola S et al (1989) Type 1 neurofibromatosis: selective expression of extracellular matrix genes by Schwann cells, perineurial cells, and fibroblasts in mixed cultures. J Clin Invest 84 (1):253–261
45. Druker BJ (2008) Translation of the Philadelphia chromosome into therapy for CML. Blood 112(13):4808–4817
46. Wu J et al (2008) Plexiform and dermal neurofibromas and pigmentation are caused by Nf1 loss in desert hedgehog-expressing cells. Cancer Cell 13(2):105–116