Neurofibromatosis and lessons for the war on cancer

*Karlyne M. Reilly**

Keywords: malignant peripheral nerve sheath tumour; neurofibroma; Neurofibromatosis; Schwann cell; Sox9

In the war on cancer, a great deal of attention is being paid to knowing the 'enemy'. It is widely believed that by understanding the driving forces underlying cancer, researchers can develop better ways to target the disease. Currently, large-scale efforts have been under taken to completely characterize molecular changes in common human cancers (http://cancergenome.nih.gov/) (Collins & Barker, 2007). However, as more is learned about cancer, the debate increases on what exactly the enemy is: cells making up the bulk of the tumour, rare tumour stem cells that can regrow the tumour, tumour microenvironment, the subset of cancer cells with metastatic potential, *etc.* Studies of the cancers associated with Neurofibromatosis type 1 (NF1) are helping to define the relationship between many of these different cell types. It is still unclear how these different enemies are related to each other and how they interact to wage cancer's war on the patient.

'If you know the enemy and know yourself you need not fear the results of a hundred battles.' – Sun Tzu, The Art of War, c. 500 B.C.

One of the greatest difficulties in fighting cancer is that pathways and mechanisms used by cancer are so closely related to pathways important for development and normal functioning. As an example, Ras signalling is important in a wide variety of normal biological functions, but serves as a key pathway in tumourigenesis. The question then arises how to kill cancer without hurting the patient, and hence the 'know yourself' becomes as important a part of winning the war on cancer as 'know the enemy.'

The familial genetic disease NF1 illustrates the difficulty of defining where self ends and the tumour enemy begins. NF1 patients carry a mutation in the tumour suppressor gene, *NF1*. *NF1* encodes the protein neurofibromin that down-regulates Ras from the active to inactive form.

> *» . . .for NF1 patients, 'normal' cells are not completely normal and may already be along a continuum of tumourigenesis, . . . «*

Many studies have shown that cells carrying one mutant copy of *NF1* are hypersensitive to growth factors, with a haploinsufficient phenotype (Gutmann et al, 1999; Wu et al, 2005; Zhang et al, 1998). This suggests that for NF1 patients, 'normal' cells are not completely normal and may already be along a continuum of tumourigenesis. NF1 patients develop neurofibromas, benign tumours of peripheral nerves. Schwann cells (SCs) have been shown to be the critical tumour cell type (Serra et al, 2000), with complete loss of wild-type *NF1*, but it is also clear that formation of neurofibromas depends on contributions from surrounding *NF1*−/+ stromal cells (Yang et al, 2008; Zhu et al, 2002).

NF1 patients develop different types of neurofibromas with different implications for patients' lives (Fig 1). Dermal neurofibromas (DNFs) are cutaneous or subcutaneous and can vary dramatically in number between patients. In extreme cases, DNFs cover the body and lead to a significant decrease in quality of life due to cosmetic disfigurement and pain or itching at the tumour site. Although the decreased quality of life for NF1 patients should not be underestimated, these benign tumours do not progress to malignancy. In contrast to DNFs, plexiform neurofibromas (PNFs) grow along internal nerve bundles and can become very large. Although benign, these tumours can be life threatening by impinging on internal organs, arteries or the central nervous system, and therefore often require surgery. Like DNFs, PNFs can cause extreme cosmetic disfigurement and pain. In contrast to DNFs, PNFs can undergo malignant transformation to malignant peripheral nerve sheath tumours (MPNSTs) in about 10% of the cases. Patients tend to have a single PNF, as opposed to the many DNFs found in patients. Given the similarities and differences between DNFs and PNFs, it is an

Mouse Cancer Genetics Program, NCI, NIH, Frederick, MD, USA.
***Corresponding author:** Tel: (+301) 846 7518; Fax: (+301) 846 7017;
E-mail: kreilly@ncifcrf.gov
DOI 10.1002/emmm.200900029

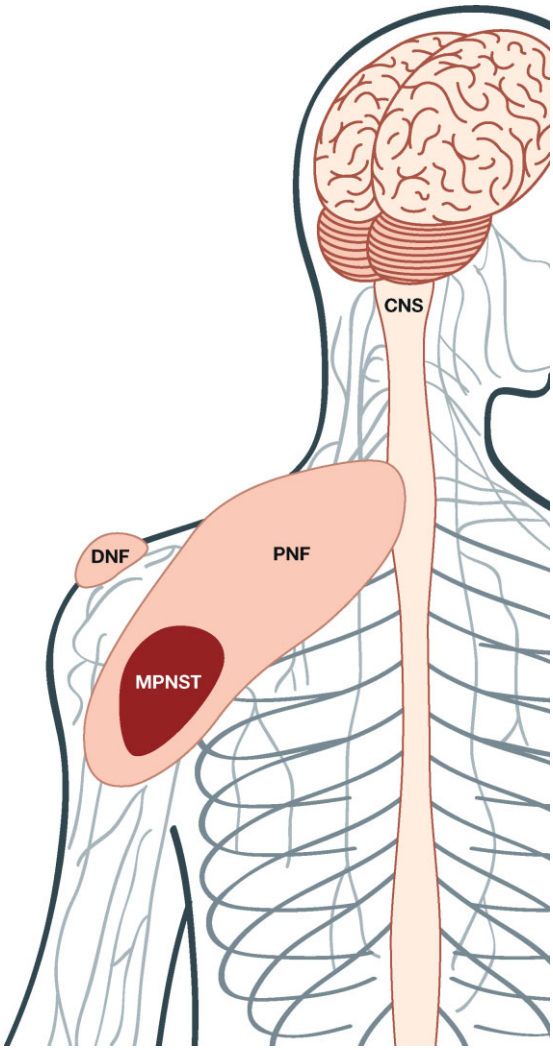

Figure 1. Different types of neurofibromas in NF1 patients: DNFs form from smaller nerves near the skin, whereas PNFs form from larger nerve bundles in the body. PNFs can transform into MPNSTs, whereas DNFs do not.

open question as to whether these two types are distinct tumours with different cells of origin along the SC lineage and different genetic alterations, or whether the differences between these tumours is due to the environmental constraints of where they initiate in the peripheral nervous system. This is particularly interesting to consider in light of their different propensities to progress to malignancy, and could have instructive implications for other cancers where one needs to determine which benign tumours will undergo malignant transformation.

» . . .tumour cells in DNF and PNF are far more similar than would have been predicted. . . «

In the article by Miller et al, 2009 (please see page 236 in this issue), the NF1 microarray consortium has addressed the question of the relationship between DNF, PNF and MPNSTs through analysis of the SC compartment. They isolated normal SCs, and SCs from DNFs and PNFs, and MPNSTs cell lines, comparing them using gene expression analysis. In addition, they compared the SC signature to the signature of the whole tumours (DNFs, PNFs and MPNSTs). Interestingly, they have found no significant differences between DNF SCs and PNF SCs, suggesting that these tumours have different characteristics due to the effects of surrounding stromal cells, local environment or developmental timing of tumourigenesis. The comparison between isolated SCs and the neurofibromas, or between cultured MPNST cell lines and MPNSTs, showed different gene expression patterns that could represent the stromal cell signature. Alternatively, as the authors note, the differences between DNFs and PNFs may be due to a very small subpopulation of cells in the tumour (cancer stem cells) that are masked in the microarray analysis. The data demonstrate that the bulk of tumour cells in DNF and PNF are far more similar than would have been predicted by observation of these physically very distinct tumours.

The article also highlights the relationship between normal development and cancer. In analysing gene expression signatures that distinguish normal, benign and malignant cell types, they found links to neurogenesis, morphogenesis, skeletal development and nervous system development. By analysing gene clusters with respect to neural crest and SC development, they found that genes associated with early stages of neural crest migration were associated with tumours, whereas genes activated later in SC development were downregulated. This highlights the importance of understanding 'self' and the normal developmental processes to better characterize the state of the tumours.

» . . .Sox9 is a promising candidate for distinguishing MPNSTs from other tumour types, . . . «

One of the clinical difficulties in dealing with MPNSTs is that these sarcomas are often highly undifferentiated and complex. The differential diagnosis of

MPNSTs from other sarcomas is often inconclusive due to a lack of tumour markers. The most commonly used marker, S100, is expressed to varying degrees by other tumour types, and is not expressed by all MPNSTs. Miller et al have found that Sox9 is a promising candidate for distinguishing MPNSTs from other tumour types, and shows differences in the number of positive cells and the degree of nuclear localization in DNFs, PNFs and MPNSTs, suggesting that this marker could aid in grading neurofibromas and MPNSTs. Finally, Miller et al demonstrate that Sox9 is functionally important for tumour cell survival, raising the possibility that Sox9 is an oncogene that could be used as a target for therapy.

In all, Miller et al has provided a detailed look at the similarities and differences between normal and tumour cell types, identifying an important marker and therapeautic target, Sox9. These data provide interesting insights into the relationship between benign quiescent tumours and benign tumours that can progress to malignancy. A better understanding of neurofibromas and MPNSTs has the potential to greatly improve the lives of NF1 patients, as well as serving as a general model for progression to be tested in other more common cancers.

The author declares that she has no conflict of interest.

References

Collins FS, Barker AD (2007) Mapping the cancer genome. Pinpointing the genes involved in cancer will help chart a new course across the complex landscape of human malignancies. Sci Am 296: 50-57

Gutmann D, Loehr A, Zhang Y, Kim J, Henkemeyer M, Cashen A (1999) Haploinsufficiency for the neurofibromatosis 1 (NF1) tumor suppressor results increased astrocyte proliferation. Oncogene 18: 4450-4459

Miller SJ, Jessen WJ, Mehta T, Hardiman A, Sites E, Kaiser S, Jegga AG, Li H, Upadhyaya M, Giovannini M, *et al* (2009) Integrative genomic analyses of neurofibromatosis tumours identify SOX9 as a biomarker and survival gene. EMBO Mol Med 1: 236-248.

Serra E, Rosenbaum T, Winner U, Aledo R, Ars E, Estivill X, Lenard HG, Lazaro C (2000) Schwann cells harbor the somatic NF1 mutation in neurofibromas: evidence of two different Schwann cell subpopulations. Hum Mol Genet 9: 3055-3064

Wu M, Wallace MR, Muir D (2005) Nf1 haploinsufficiency augments angiogenesis. Oncogene 25: 2297-2303.

Yang FC, Ingram DA, Chen S, Zhu Y, Yuan J, Li X, Yang X, Knowles S, Horn W, Li Y, *et al* (2008) Nf1-dependent tumors require a microenvironment containing Nf1+/− and c-kit-dependent bone marrow. Cell 135: 437-448

Zhang YY, Vik TA, Ryder JW, Srour EF, Jacks T, Shannon K, Clapp DW (1998) Nf1 regulates hematopoietic progenitor cell growth and ras signaling in response to multiple cytokines. J Exp Med 187: 1893-1902

Zhu Y, Ghosh P, Charnay P, Burns DK, Parada LF (2002) Neurofibromas in NF1: Schwann cell origin and role of tumor environment. Science 296: 920-922

