

# Magic but treatable? Tumours due to loss of Merlin

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## REVIEW ARTICLE

## Magic but treatable? Tumours due to loss of Merlin

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**Alterations in the NF2 gene coding for merlin cause all tumours that occur in patients suffering from neurofibromatosis type 2, all spontaneous schwannomas and the majority of meningiomas. Thus merlin's tumours are quite frequent and also numerous when inherited as part of neurofibromatosis type 2. Tumours caused by mutations in the NF2 gene are benign and thus do not respond to classical chemotherapy. Surgery and radiosurgery are only local therapies and the patients frequently require multiple treatments. This highlights the medical need to understand how merlin loss results in tumourigenesis and the need to find new systemic therapies. The benign, and therefore genetically stable and homogenous character of the tumours allows establishment of meaningful tumour models. This brings about the rather unique opportunity to both analyse the consequences of the gene defect and identify new therapeutic targets. In this review, I will first describe the phenotypes associated with 'merlin' mutations and consider differential diagnosis, in particular Schwannomatosis, for which a gene defect has been described recently. Existing therapeutic options, surgery and radiosurgery, including new data on the latter will be reviewed. Finally, I will discuss how loss of merlin leads to tumourigenesis in order to understand the rationale for emerging new therapeutic targets.**

**Keywords:** schwannoma; meningioma; ependymoma; neurofibromatosis 2; merlin

**Abbreviations:** HRS = hepatocyte growth factor-regulated tyrosine kinase substrate; Merlin = Moesin-ezrin-radixin-like protein; NF2 = neurofibromatosis 2; PAK = P21-activated kinase

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## Introduction

Mutations in the NF2 gene coding for merlin are the cause of all tumours associated with autosomal dominant inherited neurofibromatosis 2 (NF2) namely schwannomas, meningiomas, retinal hamartomas and ependymomas. Both, NF2, and the related inherited disease Schwannomatosis are characterized by a huge total tumour load. While some schwannomas in Schwannomatosis seem to be caused by mutations in the *INI1/SMARCB1* gene, most are caused by a mutation in the NF2 gene as in NF2. Mutations in the NF2 gene are also responsible for 50–60% of spontaneous meningiomas, a small proportion of spontaneous ependymomas and probably all spontaneous schwannomas. Tumours caused by NF2 mutations are thus frequent and genetically well described, thus it is relevant to try to understand their biology. Merlin (Moesin-ezrin-radixin-like protein), also called schwannomin, is a 69 kDa protein

encoded by the NF2 gene (Rouleau, 1993; Trofatter, 1993). 'Merlin's' tumours are actually caused by alterations in both gene copies and therefore loss of merlin function.

I will discuss the clinical presentation and treatment of the most frequent tumours caused by merlin loss: schwannomas and meningiomas. As only a subset of spontaneous ependymomas are caused by merlin loss, and as ependymomas are also less frequent than schwannomas and meningiomas in NF2, I will discuss ependymomas only briefly. For NF2, and for Schwannomatosis, there are specific issues due to the multiplicity of these tumours which I will discuss separately. This is of particular importance as distinguishing NF2 from Schwannomatosis clinically can sometimes be difficult. Finally, I will try to paint a picture of the common underlying pathogenesis of all merlin deficient tumours nurturing the hope of useful biomarkers, and for a molecular targeted, systemic therapy.

## Materials and Methods

### Meningiomas

It is estimated that up to 20–30% of all brain tumours are meningiomas, and the frequency in autopsies is 1.4% (Louis, 2002). The majority of meningiomas are spontaneous and there is a link between their development and exposure to ionizing radiation, however there are rare cases of familial occurrence. Meningiomas come in different histopathological types and are grouped by the WHO into grades I–III with 90% being benign.

Mutations in both alleles of the *NF2* gene, leading to loss of merlin, are the most common gene defects in meningiomas. Total 50–60% of all spontaneous meningiomas and all *NF2* associated meningiomas have mutations in the *NF2* gene. In some of the remaining 40–50% there is evidence for additional mechanisms of merlin inactivation, for example calpain-mediated proteolysis of merlin or aberrant methylation in the 5' region of the *NF2* gene (Kimura, 1998; Lomas, 2005). 'Merlin' mutations are generally assumed to be an initial gene defect in meningiomas and when tumours progress to WHO grade II and III they acquire additional genetic defects. Thus there seems to be no correlation between 'merlin' mutation and meningioma grade. 'Merlin' mutations tend to be found less, however, if the meningioma is of a meningothelial or secretory histopathological type no. (Riemenschneider, 2006; Simon, 2007).

Symptoms caused by meningiomas depend on localization and are beyond the scope of this review, they may however be asymptomatic for quite some time reviewed in (Whittle, 2004).

Total 5–15% of patients presenting with meningioma exhibit multiple meningiomas and are of particular clinical interest. Multiple meningiomas can be explained by intradural or subarachnoidal spread, or by a genetic predisposition to develop meningiomas. It is important to point out, that patients with two or more meningiomas and any *NF* associated sign e.g. juvenile lens opacity raises, could be diagnosed with presumptive or probable *NF2* due to the diagnostic criteria of *NF2* (Table 1) which has implications for genetic counselling. A population based study in Finland showed that 20% of patients with multiple meningiomas had *NF2* (Antinheimo, 2000) Evans *et al.* (2005) reported that 8% of *NF2* patients present with meningioma before developing schwannoma, however in children with multiple meningiomas the percentage that the develop additional schwannoma seems to be as high as 20%. Children should therefore be screened and followed up for other manifestations of *NF2*. In non-familial multiple meningiomas Heinrich *et al.* (2003) found that three out of seven patients had *NF2* mutations in the tumour but not in the blood. The actual mutation rate in these patients may however be higher as sensitivity of mutation detection is around 90% even if tumour tissue is used. Correct diagnosis and genetic counselling can be further complicated by the fact that adults with multiple meningiomas can be genetically mosaic for 'merlin' mutations. In those cases there is a slight risk of the offspring developing *NF2*. Identical *NF2* gene mutations in different meningiomas from the same patient would support the mosaic diagnosis. It is clear however that there are patients with multiple meningiomas but without merlin loss at all and, in summary, adults with multiple non-familial meningiomas and no other signs of *NF2* are usually not considered to have a high risk of *NF2*.

Meningioangiomas are rare and histologically different from multiple meningiomas as it seems to involve the cortex and has

more vascular involvement. However ~15% of these tumours have an association with *NF2* (Omeis, 2006).

Diagnosis and treatment of meningiomas caused by 'merlin' mutations is not different from standard treatment of meningiomas. Antinheimo *et al.* (1997) found more mitotic figures in *NF2*-associated meningiomas than in sporadic meningiomas. MR and surgery are gold standard in enlarging or symptomatic tumours, radiotherapy is recommended in malignant and unresectable or those resected incompletely (Goldsmith, 2006). Control rates with stereotactic radiosurgery are at 60–93% (Chin, 2003). Again patients with multiple meningiomas or *NF2* are important to differentiate as one should be a little more reluctant with radiotherapy in patients with multiple meningiomas because one has to take into account that these patients can have a genetic predisposition to develop meningiomas and radiation therapy may evoke the second hit resulting in an increased rate of secondary malignancies. However, benign meningiomas caused by merlin loss, singular or multiple, sporadic or familial, have potentially new molecular targets in common with other tumours caused by merlin loss which is discussed at the end of the review.

### Schwannomas

All schwannomas are caused by loss of merlin expression due to alterations in the *NF2* gene. Schwannomas are encapsulated tumours of pure Schwann cells that do not invade the nerve. Schwannomas are quite frequent and occur as part of the hereditary tumour diseases *NF2* and Schwannomatosis as well as spontaneously. It is extremely rare for a schwannoma to transform and become malignant. Schwannomas occur in different locations. Total 3–4% of all autopsies show cranial nerve schwannomas (Schneider, 1983), the vestibular nerve being the most frequent involved cranial nerve resulting tinnitus, hearing loss and vertigo. A UK survey revealed that 1 in 1000 will be diagnosed with *NF2* in their lifetime (Evans, 2005). In addition to the vestibular nerve, excepting the trigeminal nerve, other cranial nerves are rarely affected. In a Finnish study (Antinheimo, 2000) ~3% of the patients examined had multiple schwannomas in association with *NF2*, and 2% had Schwannomatosis. Schwannomas usually only cause subtle symptoms, depending on their location, and most do not cause pain (except in Schwannomatosis). Schwannomas occur on the spinal nerve roots and along peripheral nerves, with an estimated annual occurrence of 1 in 4000 (Antinheimo, 2000). Of patients with spinal schwannomas one study reported that 76% had an isolated tumour and 13% had *NF2* (Evans, 1992). When occurring sporadically, spinal schwannomas are usually only diagnosed once they become symptomatic. Their presenting features are related to their location and can include muscle weakness, sensory abnormalities, occasionally localized or radicular pain and changes in bladder function. In *NF2*, spinal schwannomas are detected more frequently because of now routine screening with spinal MRI. Spinal tumour-related symptoms are reported by 26–33% of individuals with *NF2*.

On peripheral nerves, schwannomas occur as fusiform swellings usually separate from the overlying skin. In *NF2*, tumours may also occur intracutaneously as a plaque-like lesion that is thicker and darker than the overlying skin and often more hairy (Evans, 1992). In Schwannomatosis these plaque lesions do not occur. The incidence of peripheral tumours is almost certainly underestimated, as in *NF2* close to 70% of individuals have some evidence of peripheral involvement (Evans, 1992; Sperfeld, 2002),

**Table 1** Clinical criteria for NF2

Manchester criteria <sup>a</sup>	
A.	Bilateral vestibular schwannomas
B.	First-degree family relative with NF2 and unilateral vestibular schwannoma or any two of: meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lenticular opacities
C.	Unilateral vestibular schwannoma and any two of: meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lenticular opacities
D.	Multiple meningiomas (two or more) and unilateral vestibular schwannoma or any two of: schwannoma, glioma, neurofibroma, cataract
NNFF criteria <sup>b</sup>	
A.	Confirmed or definite NF2
1.	Bilateral vestibular schwannomas
2.	First-degree family relative with NF2 and unilateral vestibular schwannoma at less than 30 years of age or any two of: meningioma, schwannoma, glioma, juvenile lens opacity (posterior subcapsular cataract or cortical cataract)
B.	Presumptive or probable NF2
1.	Unilateral vestibular schwannoma at less than 30 years of age and at least one of: meningioma, schwannoma, glioma, juvenile lens opacity (posterior subcapsular cataract or cortical cataract)
2.	Multiple meningiomas (two or more) and unilateral vestibular schwannoma at less than 30 years of age or at least one of: schwannoma, glioma, juvenile lens opacity (posterior subcapsular cataract or cortical cataract)

<sup>a</sup>In the Manchester criteria, 'any two of' refers to two individual tumours or cataract, whereas in the other sets of criteria, it refers to two tumour types or cataract.

<sup>b</sup>For the purposes of this study, the NNFF criteria for confirmed or definite NF2 and or presumptive or probable criteria were considered to be equivalent.

and Antinheimo *et al.* (2000) reported a 1 in 2000 lifetime risk of developing cutaneous schwannomas.

For reliable detection of vestibular schwannomas thin (3 mm) and overlapping MRI sections at the brainstem are now standard. The usefulness of audiological follow-up for VS has been questioned (Masuda, 2004). Modern imaging as a means to follow patients has come into sharper focus, despite the fact that, speech and pure tone audiometry are necessary to detect clinically relevant deterioration of hearing (Mautner, 2002; Slaterry, 2004). A study using two-dimensional morphometric MRI analysis of vestibular schwannoma, showed a pattern of slow tumour growth which declines with patient age. However, there was some variability in the two-dimensional measurements indicating that three-dimensional volumetric analysis may be a better tool for future studies of this type (Herwadker, 2005).

As schwannomas are benign tumours that respond poorly to classical chemotherapeutic regimes, surgery and increasingly radiosurgery are the current standard therapies. A number of surgical approaches can be employed and hearing preservation is possible in selected cases. Depending on extent of preoperative symptoms, tumour size, location, surgical approach and experience of the centre, hearing loss and facial nerve involvement can be as high as 50% resp. 40% function (Samii, 1997; Ho, 2002). An alternative to surgery is stereotactic radiation treatment; local tumour control rate seems to be over 90% and importantly hearing loss and involvement of facial nerves seems to be below 5% (Rowe, 2003; Combs, 2005).

In NF2, results are slightly worse with lower hearing preservation reduced to around 68% and tumour control around 80% (Rowe, 2003; Mathieu, 2007). However, longer follow-up periods are needed on patients who have had radiosurgery to compare with surgery. These follow-up studies should also carefully analyse the possibility of new tumours and malignant transformation especially in NF2 patients after radiotherapy (Evans, 2006).

Another treatment approach is to 'watch and wait'. As schwannomas are very slow-growing tumours many patients, especially older ones never require treatment. This also calls into question the apparent 'tumour control' of radiation treatment in some patients. Resection of non-vestibular cranial nerve schwannomas can result in significant side effects. In general, non-vestibular schwannomas in NF2 do not grow inexorably and can be left alone.

Very few studies have addressed the management of spinal schwannomas. The clinical course of patients harbouring multiple spinal tumours is varied, with some tumours growing slowly and remaining asymptomatic, whilst others exhibit rapid growth with resultant deterioration in neurological status. Larger tumour size at presentation may be a guide to the likelihood of future progression (Dow, 2005). Thus there is variation in management of these tumours, with some authors advocating surgery for cord compression in the absence of symptoms, whilst others advocate watchful waiting except when tumours show rapid growth or symptomatic progression (Kim, 1989; Klekamp, 1998).

## Ependymomas

Ependymomas account for 2–5% of all intracranial tumours and are thus the least frequent of the tumours discussed here. Approximately 29–38% of ependymomas show loss of merlin expression (Gutmann, 1997; Lamszus, 2001; Rajaram, 2005). Loss of merlin expression is probably more common in spinal ependymomas and does not significantly vary with tumour grade (Rajaram, 2005). Clear cell ependymoma, a rare histopathological variant, shows merlin loss less frequently than other ependymoma types (Fouladi, 2003). It seems clear that there are significant number of ependymomas that are caused by other gene defects and good candidates are other protein 4.1 family members (Rajaram, 2005). A recent multi-centre French study demonstrated that supratentorial ependymomas seem to be of higher grade than

infratentorial. Complete resection being the gold standard, the authors also showed that in incompletely resected ependymomas of low-grade adjuvant radiotherapy prolonged the progression free survival (Metellus, 2007).

## NF2

NF2 is caused by mutations in the *NF2* gene on chromosome 22 and is inherited in an autosomal dominant manner. Fifty percent of cases however arise from *de novo* mutations, and thus no family history is apparent. NF2 has a birth incidence between 1 in 25 000 and 1 in 30 000 (Evans, 2005) and is characterized by the development of schwannomas, as well as meningiomas, ependymomas, posterior subcapsular lenticular opacities and retinal hamartomas. Schwannomas are the most frequent tumours, however 50–60% of NF2 patients will also develop meningiomas and 6% ependymomas and spinal MRI also detects spinal tumours in 67–90% of patients (Mautner, 1995; Dow, 2005).

The clinical presentation of schwannomas and meningiomas has been described previously. Importantly, these tumours are numerous in NF2. There is evidence for histological differences between NF2 related and spontaneous schwannomas. Both the multiplicity and the possibly different histology influence treatment decisions. An additional clinical feature of NF2 is the occurrence of neuropathies (Evans, 1999; Sperfeld, 2002). It is possible that tumourlets around peripheral nerves at exits in bony foramina may contribute to asymmetric peripheral neuropathy in some patients. However, haploinsufficiency of the *NF2* gene might cause NF2-related polyneuropathies (Hanemann, 2007).

The type of constitutional mutation in the *NF2* gene influences the disease severity with nonsense and frameshift mutations that cause protein truncation confer the worst phenotype (Parry, 1996; Rutledge, 1996; Baser, 2004). A major implication for severity is genetic mosaicism. Total 25–30% of *de novo* patients are mosaic (Kluwe, 2003; Mohyuddin, 2003). In more than half of these patients the underlying mutation can only be detected by analysing multiple tumours from the same person. Although mosaic disease is relatively mild and the chance of transmission is small if the mutation is not detected in blood lymphocytes, affected offspring will contain the mutation in all their cells and be more severely affected.

Since NF2 patients also have a higher tumour recurrence rate, they need to have MRI scans of the cerebellopontine angle on a regular basis (Evans, 2005). NF2 patients also need additional MRI scans of the spinal canal initially to define the tumour load and further scans once they become symptomatic. Whole body MRI is sometimes used to estimate total tumour load in neurofibromatosis and Schwannomatosis patients, with many investigators using fat suppressed STIR sequences to detect schwannomas on whole body MRI. Individuals at 50% risk should be screened for tumours clinically and with MRI of head starting around 10–14 years, depending on the severity of the disease in the family (Evans, 2005). It seems that in children ocular manifestations are more frequent than in adults, which needs to be kept in mind when examining families (Evans, 1999). Genetic testing for mutations detects about 60% of mutations in isolated cases and 90% in families with more than one affected patient. Testing at risk individuals at around 10–14 years of age can identify the majority of non-affected offspring. Analysis of tumour material will enhance the detection rate in isolated patients by detecting the mosaic mutation (Mohyuddin, 2003).

In the absence of a mutation test MRI screening of at risk patients should probably be continued until the age of 30, particularly in the later onset, mildly affected families. After 30 years, this could probably be reduced to screening once every three years for the presence of VS.

The important difference to merlin's spontaneous tumours is that they are numerous in NF2 implying difficult therapeutic decisions. The mere presence of a tumour however does not indicate it needs to be removed. Thus the decision when and which tumour to treat is an important issue in NF2 and one should try to maintain quality of life and balance that with multiple surgeries. This again underlines the need for a systemic treatment. Outcomes in NF2 are usually worse than for sporadic VS. The life span in NF2 is substantially shortened, mostly caused by meningioma disease (Baser, 2002), although many NF2 patients actually die due to swallowing problems (Slattery, WH, personal communication).

As mentioned when discussing schwannomas, radiotherapy including radiosurgery is probably less successful in NF2 than in spontaneous schwannomas. Malignant transformation occurs in a small but significant minority and tumour control rates may diminish to 60% (Baser, 2000; Rowe, 2003). This may be due to the fact that in NF2 the tumours may arise at multiple sites at the cerebellopontine angle. Thus peripheral tumour parts may receive submaximal radiation dose.

NF2 patients should be managed in specialty centres (Evans, 2005) with a permanent staff of a neurosurgeon, otolaryngologist, neurologist, geneticist, nurse and audiologist. Patients who are deaf can be offered brain stem implant (Colletti, 2006), although hearing function lags behind cochlear implants. Nonetheless, if the cochlear nerve is intact patients can be rehabilitated with a cochlear implant (Evans, 2005).

## Schwannomatosis

Recently, a clinically and molecularly distinct form of hereditary disease with multiple schwannomas, Schwannomatosis, has been described. It is important to differentiate Schwannomatosis from NF2. Schwannomatosis is usually sporadic, but families exhibiting autosomal dominant transmission do exist (Evans, 1997; MacCollin, 2005). Schwannomatosis does usually not shorten the lifespan, but quality of life can be affected by the tumour-induced pain. Patients suffering from schwannomatosis have multiple schwannomas, but do not have bilateral VS that are characteristic of NF2. Occasionally they develop meningiomas. Ependymomas and ocular abnormalities have, however, not been described in schwannomatosis. Schwannomatosis is characterized by the development of multiple peripheral nerve and spinal nerve root schwannomas but absence of plaque like intracutaneous lesions found in NF2 (clinical criteria are shown in Table 2). Unlike in NF2, motor symptoms seem to be less frequent in schwannomatosis. The frequency of schwannomatosis is not clear but was suggested to be nearly equivalent to NF2 (Antinheimo, 2000; MacCollin, 2005), however according to the Finnish study the incidence for schwannomatosis was estimated 1 in 134 000 (Antinheimo, 2000). Genetically schwannomatosis seems to be heterogeneous. A Dutch group recently found a mutation in the *IN11/SAMRCB1* gene in the germ line and in the tumours of schwannomatosis patients (Hulsebos, 2007) that presumably can be found in ~20% of schwannomatosis patients (Evans DG, personal communication). Some schwannomatosis patients exhibit NF2 mutations in their tumours along with loss of the wild-type allele.



**Table 2** Proposed clinical criteria for schwannomatosis (Baser, 2006; MacCollin, 2005)

Definite	Possible
Age >30 years and two or more non-intradermal schwannomas, at least one with histologic confirmation and no evidence of vestibular tumour on high-quality MRI scan and no known constitutional NF2 mutation	Age >30 years and two or more non-intradermal schwannomas, at least 1 with histologic confirmation and no evidence of vestibular tumour on high quality MRI scan and no known constitutional NF2 mutation
or One pathologically confirmed non-vestibular schwannoma plus a first-degree relative who meets above criteria	or Age >45 years and two or more non-intradermal schwannomas, at least one with histological confirmation and no symptoms of eight nerve dysfunction and no known constitutional NF2 mutation  or Radiographic evidence of a non-vestibular schwannoma and first degree relative meeting criteria for definite

Segmental: meets criteria for either definite or possible schwannomatosis but limited to one limb or five or fewer contiguous segments of the spine.

Although it is usually possible to distinguish schwannomatosis from NF2, mosaic NF2 can often present with clinical features consistent with schwannomatosis criteria (Mohyuddin, 2003; Murray, 2006), thus there still may be clinical overlap between schwannomatosis and neurofibromatosis. It has hence been suggested to diagnose schwannomatosis only when patients do not meet diagnostic criteria for NF2 and have no constitutional NF2 mutation (Baser, 2006; Murray, 2006). Patients with an inherited schwannomatosis disease should also have spinal axis MRI.

In summary, both NF2 and schwannomatosis are characterized by early onset and by the occurrence of multiple tumours. The early tumour burden and tumour load are the main therapeutic dilemmas.

The current treatment of surgery and radiosurgery are, *prima vista*, aiming at a single specific tumour. In patients suffering from a hereditary disease it is often difficult to decide whether to treat the tumours and, if this course is chosen, which tumour should be treated first. Furthermore, tumours in NF2 patients seem to have a reduced tumour control rate with both therapies, at least when comparing NF2 schwannomas to spontaneous schwannomas. In order to understand the rationale for emerging new therapeutic targets we need to discuss how merlin functions normally and how merlin loss may be involved in tumourigenesis. New therapeutic targets in merlin’s tumours are certain growth factor receptors, the Rac/PAK signalling pathway, the Pi3K/Akt signalling pathway and the Ras/Raf/Mek signalling pathway. Luckily, some of these targets have been investigated in other cancers and therefore drugs targeting these pathways already passed phase I trials successfully. I will explain what each of these targets is and why they are promising for merlin’s tumours.

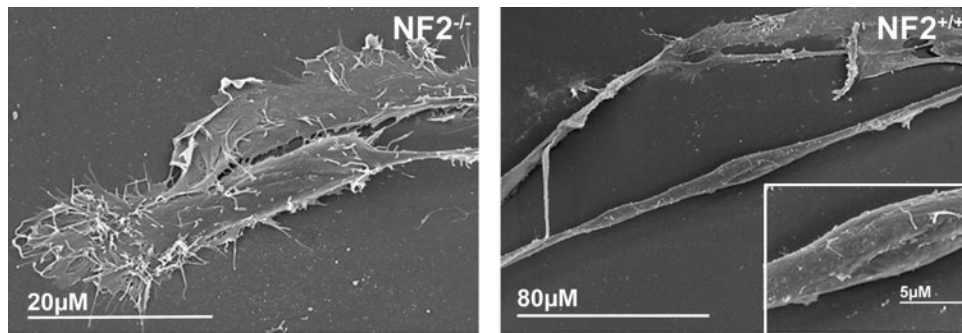
The neurobiology of merlin

‘Merlin’ shows close homology to the family of ERM (*ezrin*, *radixin*, *moesin*) proteins, which act as membrane cytoskeletal linkers; highest homology is found in the N-terminal globular FERM domain. Merlin has also been demonstrated to act as a tumour suppressor (Gautreau, 2002). There are different splice forms of ‘merlin’, however the most prominent are isoforms 1 and 2 which differ only in the very C-terminal end. Isoform 1 consists of exons 1–15 and exon 17 coding for 595AA while isoform 2 is comprised of exons 1–16 resulting in a 590AA protein. It is thought that isoform 1 can adopt a closed conformation on dephosphorylation and then acts as a tumour suppressor, although this is not finally settled yet (Sherman, 1997).

Although merlin is expressed in all tissues affected in NF2, it is also expressed in unaffected tissues. This is, however, a frequently encountered problem in human genetics, and may be caused by other proteins taking up merlin’s role in tissue not affected by merlin loss or by differential expression of relevant merlin interaction partners. More specifically, merlin is expressed in a variety of tissues during embryonic development, (Akhmetyeva, 2006), in adulthood expression has been demonstrated in the retina (Chan, 2002), lens (Claudio, 1997), testis, ovary, adrenal gland and neuronal tissue (Gutmann, 1995). There are conflicting data on expression in other tissues, however, expression in neuronal tissue includes both Schwann cells as well as neurons (Gronholm, 2005).

On a subcellular level merlin is primarily found in actin-rich cellular protrusions like the leading edge of the cell (lamellipodia and membrane ruffles) (Gonzalez-Agosti, 1996), and in cell–cell and cell–matrix contact sites co-localizing with focal adhesion and focal complex proteins (Fernandez-Valle, 2002; Lallemand, 2003). Interestingly, a Finnish group has shown that merlin also shuttles to the nucleus in a cell cycle-dependent manner (Muran, 2005). Many merlin interaction partners have been identified in the last couple of years including transmembrane proteins, scaffolding/adaptor proteins, signalling molecules/kinases, cytoskeletal proteins, various other proteins and proteins of yet unknown function [Kissil J, *Merlin*, UCSD Nature-Molecular-Pages (2006), doi:10.1038/mp.a001631.01; Hanemann CO in Monograph in Human Genetics, editor D. Kaufmann in press]. Until now only some of these merlin interaction partners have been examined in cells or tissues that are actually affected by ‘merlin’ mutations in humans, but nevertheless these interactions provide important insight into merlins function. For some of the interaction partners it has been shown or speculated that merlin exerts its role as a tumour suppressor through the interaction with binding partners. Importantly, some interaction partners are components of multi-molecular signalling pathways and thus part of a bigger picture discussed below.

Different *in vitro* models using a variety of cell lines and mouse models have been instrumental in understanding how merlin loss leads to tumourigenesis (Huynh, 1996; Sherman, 1997; Giovannini, 2000; McClatchey, 2000; Kalamarides, 2002). As the cell lines used have, however, different underlying mutations, one has to be careful to transfer results one to one to explain phenotypes of cells where the primary mutation is merlin loss. Some of the mouse models, which used a conditional knockout technique applying the flox technology reflect parts of the human disease (Giovannini, 2000; Kalamarides, 2002) others show an interesting but different phenotype (McClatchey, 1997, 1998).



**Fig. 1** Scanning electron microscopic pictures showing schwannoma (NF2<sup>-/-</sup>) and normal Schwann cells (NF2<sup>+/+</sup>). Schwannoma cells are more spread, show multiple membrane extensions everywhere, and fail to contact each other in an orderly fashion. Normal Schwann cell are less spread, bipolar with few membrane extensions.

One explanation as to why mouse models have a slightly different phenotype from that found in human, is that some merlin interaction partners identified in different rodent cells and cell lines are not found in human Schwann cells. Another reason for the differing phenotypes between mice and men might be that the timing of the mutation in the human disease is different from that in the animal models. As it has been shown on many occasions that there is no merlin expression in tumours caused by ‘merlin’ mutations, it is generally assumed that ‘merlin’ loss or inactivation is the starting point. Mathematical modelling (Woods, 2003) and the fact that conditional knockout mice develop schwannomas late in life suggest a third hit. However, so far, no additional genetic or epigenetic events have been found in NF2 patients in tumours caused by merlin loss.

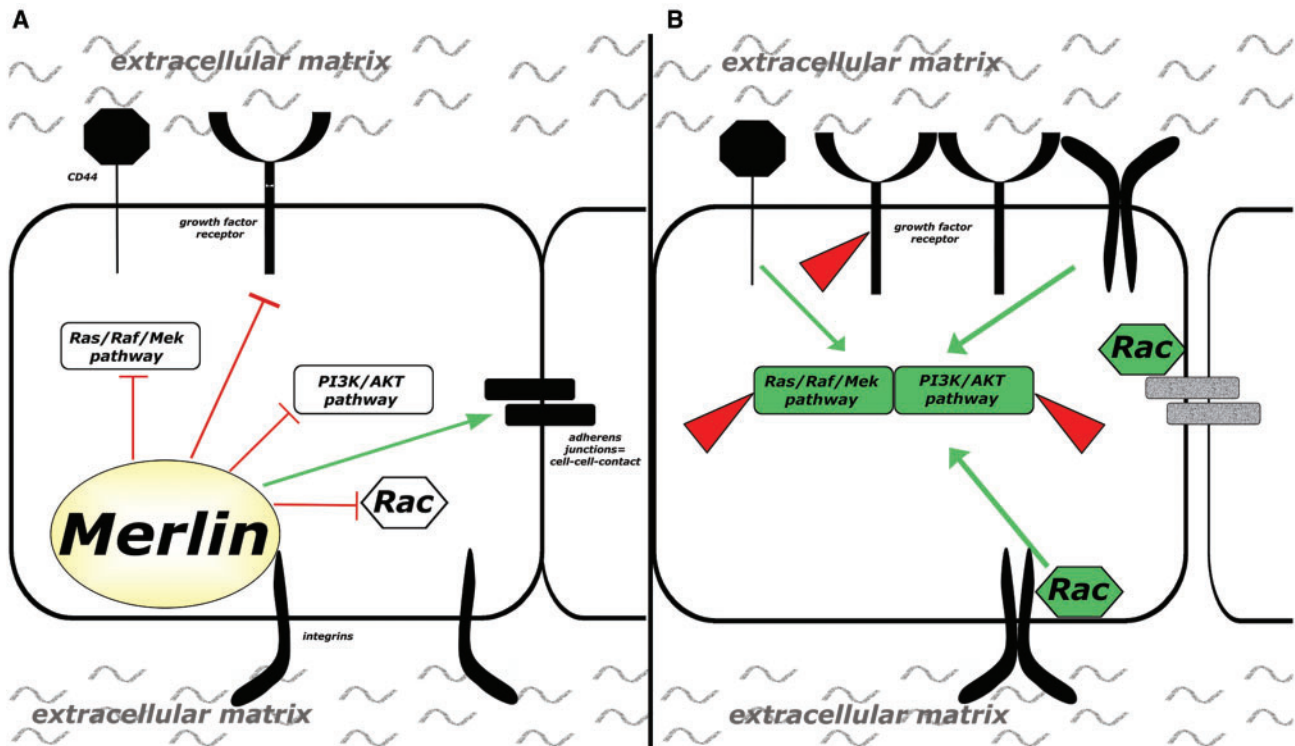
By comparing primary human schwannoma cells with normal human Schwann cells as an *in vitro* model, it was shown that merlin-deficient human cells show slightly increased proliferation and increased cell spreading (Pelton, 1998; Rosenbaum, 1998) reversible on the reintroduction of ‘merlin’ (Schulze, 2002), increased adhesion to extracellular matrix (Utermark, 2003), slightly decreased apoptosis (Utermark, 2005) and altered cytoskeleton (Pelton, 1998; Utermark, 2005; Flaiz, 2007). Figure 1 shows two spreading and ruffling schwannoma cells (NF2<sup>-/-</sup>) compared to an elongated slim normal Schwann cell (NF2<sup>+/+</sup>).

### So what does all this mean in regard to possible disease mechanism?

It is impossible to mention all hypotheses on how merlin loss could lead to tumourigenesis, thus only those which have accumulated evidence through multiple publications and are of potential future therapeutic relevance will be discussed here.

Before discussing individual theories one has to consider that many theories are based on *in vitro* comparison of NF2<sup>-/-</sup> and NF2<sup>+/+</sup> cells in confluent as well as subconfluent conditions, whichever reflect the *in vivo* situation best. In confluent cultures of NF2<sup>-/-</sup> cells, ‘merlin’ expression is increased and these cells show loss of contact inhibition (Shaw, 1998; Rosenbaum, 2000; Lallemand, 2003). In subconfluent cultures NF2<sup>-/-</sup> cells, including primary human schwannoma cells, show slightly increased proliferation and increased adhesion to the extracellular matrix (Pelton, 1998; Rosenbaum, 1998; Utermark, 2003).

Regarding adhesion it is interesting to note that merlin binds to adhesion molecules such as  $\beta 1$  integrin and layllin and to molecules which are part of the focal adhesion complexes such as paxillin and focal adhesion kinase (Fernandez-Valle, 2002; James, 2004; Bono, 2005). Thus it makes sense that merlin-deficient cells show deregulated adhesion to extracellular matrix, as shown in schwannoma cells (Utermark, 2003). This might explain why schwannoma cells seem not to properly myelinate axons but instead build pseudomesaxon around the extracellular matrix already described in the older electron microscopic literature (Dickersin, 1987). Additionally, merlin seems to be directly involved in cytoskeletal organization that is highly relevant in myelination. Not only does it bind actin and tubulin (Muranen, 2007) but it also inhibits the actin nucleation promotion factor N-WASP, thereby regulating actin polymerisation (Manchanda, 2005). This implies that merlin loss would lead to more actin-rich cellular protrusions, which has been clearly demonstrated in human schwannoma cells (Flaiz, 2007). The first pathway which was thought to be of potential interest as a therapeutic target is thus a signalling pathway involved in adhesion, cytoskeleton regulation and in merlin phosphorylation. P21-activated kinase (PAK), downstream of the small GTPases Rac and Cdc42, has been shown to phosphorylate merlin (Sherman, 1997; Xiao, 2002; Kissil, 2003; Alftan, 2004). In a negative feedback-loop merlin inhibits Rac and PAK activation (Shaw, 2001; Kaempchen, 2003; Kissil, 2003; Hirokawa, 2004), and inhibits Rac recruitment to the membrane (Okada, 2005). Thus when merlin is lost, Rac is activated and recruited to the membrane where it is possibly further activated by integrins, which have been shown to be overexpressed in schwannomas both *in vitro* and in sections (Utermark, 2003). Rac at the membrane exerts its function in cytoskeletal and adhesive structures. Supporting this hypothesis merlin loss leads to activated Rac at the membrane in human schwannoma cells (Kaempchen, 2003) and importantly this GTPase activation is non-localized and long-lasting (Nakai, 2006; Flaiz, 2007). One could therefore postulate that NF2<sup>-/-</sup> schwannoma cells are non-polarized, in contrast to the highly polarized normal Schwann cells (see also Fig. 1), and thus fail to myelinate the axon. Rac/PAK pathway inhibitors have been successfully tried in *in vitro* models and reversed pathological adhesion, membrane extensions and proliferation of schwannoma cells (Pelton, 1998; Hirokawa, 2004; and unpublished data), however, no pharmacological Rac1 or PAK inhibitor is yet available to the author’s knowledge.



**Fig. 2** Schematic diagram of merlin's role in tumourigenesis.

Looking at contact inhibition, NF2<sup>-/-</sup> cells show impaired cell-cell contact due to destabilised adherens junctions (Lallemand, 2003; and unpublished data). Merlin's role in stabilizing adherens junctions might involve the PDZ protein called erbin, found to bind indirectly to merlin (Rangwala, 2005). In confluent cells dephosphorylated merlin also binds the transmembrane hyaluronan receptor CD44 and acts as a growth inhibitor (Morrison, 2001). Furthermore, merlin inhibits contact inhibition by suppressing Rac recruitment to the membrane at confluency (Okada, 2005). CD44 and integrins are also believed to act as co-receptors for growth factor tyrosine kinase receptors, further prompting the study of the signalling pathways downstream of most of these receptors, particularly the Ras–Raf–Mek–Erk and the PI3–Kinase–Akt pathway, both well known in oncogenesis (Fraenzer, 2003; Lim, 2003; Rong, 2004; Chadee, 2006; Lim, 2006; Morrison, 2007). Merlin has been shown to inhibit the Ras–Raf–Mek–Erk pathway at different levels (Lim, 2006) and merlin inhibits Ras and Rac activation upstream of the Raf–Mek–Erk after growth factor stimulation (Morrison, 2007). It is interesting that merlin is inhibiting Ras as this points out a parallel to NF1, where the gene mutated is a Ras inactivator (Ras-Gap).

In the context of growth factor signalling an interesting finding is that merlin interacts with hepatocyte growth factor-regulated tyrosine kinase substrate (HRS). HRS is a regulator of tyrosine kinase trafficking to the degradation pathway and an inhibitor of the STAT pathway. Merlin requires HRS to inhibit the STAT pathway and acts as a growth suppressor via HRS (Scoles, 2002, 2005). Thus there is accumulating evidence that merlin seem to interact with pathways downstream of growth factors.

An attractive, although yet unproven, hypothesis is that merlin is involved in the degradation of growth factors. This is supported

by the fact that merlin binds ebp50 (Murthy, 1998) and erbin (Rangwala, 2005) both shown to be relevant in growth factor distribution. There is now increasing evidence that one finds overexpression of certain growth factor receptor receptors like PDGF-R, ErbB2 and ErbB3 in merlin's tumours.

## Conclusions

In summary, there is accumulating evidence that merlin has an important role in the coordination of two relevant and interdependent processes which are cellular adhesion and growth factor receptor response. The role of 'merlin' in these different pathways is roughly summarized in Fig. 2.

It is thus perfectly reasonable that the above mentioned pathways are targeted in merlin's tumours with compounds like herceptin, inhibitors of the Ras/Raf/Mek [e.g. Sorafenib (Bayer), PD325901 (Pfizer)] and Pi3K–Akt [e.g. OSU3013 (AstraZeneca), Rapamycin] pathways or a PDGF-R inhibitor (e.g. Sorafenib) all of which have passed Phase I trials in other diseases.

Interestingly, an Akt inhibitor and a Ras/Raf/Mek/Erk pathway inhibitor have recently successfully been tried in a disease models for schwannoma where they clearly reduce tumour cell growth. Experiments with growth factor receptors antagonists are underway. By carefully reviewing the literature on meningiomas from the time before 'merlin' mutations could be detected, and therefore meningiomas without 'merlin' mutation were included, one finds that meningioma cells growth can be inhibited by



antagonizing the IGF1 pathway (McCutcheon, 2001) and by PDGFR antagonist (Todo, 1996). Thus I believe it will not be long before some of those drugs will be in phase II trials in order to treat merlin's tumours. As these tumours are genetically defined and as there is a medical need. Merlin's tumours should be a prime target of such new drugs. However, as it seems from the experimental data that there may be more than one pathway involved in tumourigenesis following merlin loss it is likely that combination therapy will be needed to treat these tumours efficiently.

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