Pathol. Oncol. Res. (2013) 19:365–373
DOI 10.1007/s12253-013-9644-y

REVIEW

Merlin, the NF2 Gene Product

Nives Pećina-Šlaus

Received: 13 November 2012 / Accepted: 17 April 2013 / Published online: 12 May 2013

Abstract Merlin, the protein product of *NF2* gene, is one of the most versatile tumor suppressors capable of integrating different mechanisms that regulate cell proliferation, motility, survival and signaling pathways underlying and governing those mechanisms. Merlin is considered a member of the band 4.1 families of cytoskeleton-associated proteins also called ERM family and acts as tumor suppressor. The main cause for transformation of Schwann cells into schwannomas is credited to the inactivation of the neurofibromin 2 (*NF2*) gene and the consecutive loss of its protein merlin. Recent scientific advances improved our understanding of pathogenic mechanisms involving *NF2* gene. The present review brings genetic properties of *NF2* gene, molecular characteristics of merlin, summarizes mutational spectra and explains merlin's multifunctional roles regarding its involvement in neurofibromatosis associated tumorigenesis.

Keywords *NF2* gene · Merlin · Schwannoma · Neurinoma

Introduction

The *NF2* gene was identified in 1993 [1–3], and the protein it codes was named merlin not after the mythological sorcerer, but as an acronym of the first letters of moesin-, ezrin-, radixin-like protein, being a part of the band 4.1 families of cytoskeleton-associated proteins also called ERM family of proteins. Merlin acts as tumor suppressor and its inactivation leads to the loss of the protein. Tumors evolved due to the loss of merlin, schwannomas, meningiomas, hamartomas and ependymomas, can occur as sporadic entities or as part of autosomal dominant syndrome—neurofibromatosis type 2 (NF2). Recent research on *NF2* gene [4–7] demonstrates that merlin is a tumor suppressor that is capable of modulating a wide range of signaling pathways that influence cell growth, motility and apoptosis. It interacts with cell-surface proteins, proteins involved in cytoskeletal dynamics and proteins involved in regulating ion transport. Merlin-lacking cells are also known to contain defective adherens junctions, thus connecting it to the loss of contact inhibition.

N. Pećina-Šlaus (✉)
Laboratory of Neurooncology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Šalata 12, 10000 Zagreb, Croatia
e-mail: nina@mef.hr

N. Pećina-Šlaus
Department of Biology, School of Medicine, University of Zagreb, Šalata 3,
10000 Zagreb, Croatia

Gene and Protein

The *NF2* gene resides on chromosome 22, band 22q12.2, more precisely from nucleotide 29999545 to 30094589, with total length of 95,045 nucleotides (according to the search and retrieval system used at National Centre for Biotechnology Information (US) for all of the major databases including nucleotide and protein sequences—Entrez nucleotide) [8]. It was discovered independently by two groups by positional cloning and loss of heterozygosity studies [1–3]. *NF2* gene consists of 17 exons with alternative splicing that produces two major isoforms. The exons are shown in Table 1.

NF2 homologous genes are confined to metazoans including Drosophila melanogaster (*Mer*) and Caenorhabditis elegans (*nfm-1*) and the homology of their coding sequences is very much conserved across species, with 98.49 % amino acid similarity to mouse protein sequence, and 50.87 % to *Drosophila* [9].

The protein merlin also called schwannomin has molecular weight of 65 to 70 kDa [10]. The protein is expressed ubiquitously in human tissues and also in a variety of embryonic tissues. In adult tissues, significant expression is found in Schwann cells, meningeal cells, lens and nerve. Its expression in neuronal tissue includes both Schwann cells but also neurons [11]. *NF2* gene consists of 17 exons with alternative

Table 1 Exons of the *NF2* gene, nucleotide position and length of each exon

Exon	Total range	Total length
1	5 001–5 557	557
2	38 196–38 321	126
3	40 535–40 657	123
4	43 647–43 730	84
5	56 102–56 170	69
6	57 039–57 121	83
7	59 634–59 709	76
8	62 650–62 784	135
9	66 435–66 509	75
10	69 778–69 891	114
11	73 271–73 393	123
12	74 714–74 931	218
13	76 281–76 386	106
14	79 641–79 768	128
15	82 884–83 046	163
16 (16a + 16b)	84 465–85 360	896
17	96 197–100 045	3849

Adapted from http://www.ncbi.nlm.nih.gov/; http://www.ncbi.nlm.nih.gov/gene/4771

splicing that produce two major isoforms. Isoform 1 (I) consists of exons 1 to 15 and exon 17 and codes for 595 amino acid protein, while isoform 2 (II) encompasses exons 1 to 16 and codes for a 590 amino acid protein. The isoforms differ only in the very C-terminal end [4, 11]. Two predominant isoforms but also a number of minor isoforms are produced by alternatively spliced transcripts. According to UniProtKB/Swiss-Prot [12] the isoforms are expressed specifically as follows: isoforms 1 (I) and 2 (II) are predominant, isoform 4, isoform 5 and isoform 6 are expressed moderately, isoform 8 is found at low frequency. Isoform 7, isoform 9 and isoform 10 are not expressed in adult tissues, with the exception of adult retina expressing isoform 10. Isoform 9 is faintly expressed in fetal brain, heart, lung, skeletal muscle and spleen. Fetal thymus expresses isoforms 1, 7, 9 and 10 at similar levels.

The protein product of *NF2* gene is different from ordinary tumor suppressor gene products. The NF2 protein shows significant sequence similarity to members of the band 4.1 superfamily of cytoskeleton-associated proteins called moesin-, ezrin-, radixin-like or short ERM proteins [10]. Let me explain briefly the biochemical and molecular characteristics of this protein family and its similarities and differences from merlin. The highest homology is found in the N-terminal globular FERM domain (Four-point-one, Ezrin, Radixin, and Moesin). C-terminal domain of those proteins and merlin is less conserved, where actin binding site in ERM proteins, but not merlin is located. However, merlin contains an unconventional actin-binding site located in its NH_2 terminus [4]. Carboxy terminal ERM-binding domain or C-ERMAD is shared by both merlin and ERM proteins, while alpha-helical region is in the central part of all these proteins and it allows flexing which facilitates intramolecular association [13]. The activity of mentioned proteins is regulated by intramolecular (head-to-tail) binding between FERM and C-ERMAD domains. When bound the conformation is termed as closed and the actin-binding site within the C-ERMAD domain is masked. This conformation renders ERM proteins in an inactive state [14]. Phosphorylation of critical threonine within the C-terminal domain would break up this head-to-tail interaction and render ERM proteins in their active form. Like other ERM proteins, merlin can form a C-terminal tail-FERM interactions and switch between open and closed conformation which regulates vastly its function. The conformations are also carried out through changing phosphorylation status, but this time phosphorylation on serine 518 in merlin's C-ERMAD domain is involved. The so called head-to-tail interaction is upon phosphorylation of Ser518 disrupted, but now the difference arrives, namely, merlin's open conformation is inactive, while his closed conformation renders the molecule active (Fig. 1). The phosphorylation can be accomplished by p21-activated kinases (PAKs) and c-AMP dependent protein kinase A [15]. It is known that only Ser518 hypophosphorylated (active) merlin functions as tumor suppressor. It has also been demonstrated that merlin can form homodimers and heterodimers with other members of the ERM family. It has been also shown that above mentioned intramolecular interactions are only happening on isoform 1, while isoform 2 is believed to be incapable of forming this head-to-tail interaction, but rather remains locked in open conformation, and thus incapable of acting as tumor suppressor protein. The exceptional conservation of the NF2 coding sequence across species suggests conservation of function too. Experiments have demonstrated that the human protein can function properly in both the mouse and Drosophila [9].

Merlin Is a Multifunctional Protein

As is usual with multifunctional proteins, merlin definitely being one of them, they integrate different mechanisms that regulate cell proliferation, motility, survival and signaling pathways underlying and governing those mechanisms.

To start from the beginning I will describe merlin's function in developmental circumstances. Merlin plays important roles during embryogenesis. Experiments involving knockout animals showed that homozygous NF2−/− knockout mice embryos lack extraembryonic ectoderm and die even before gastrulation [16] indicating merlins role at very early stages. Mosaic embryos that only in part posses

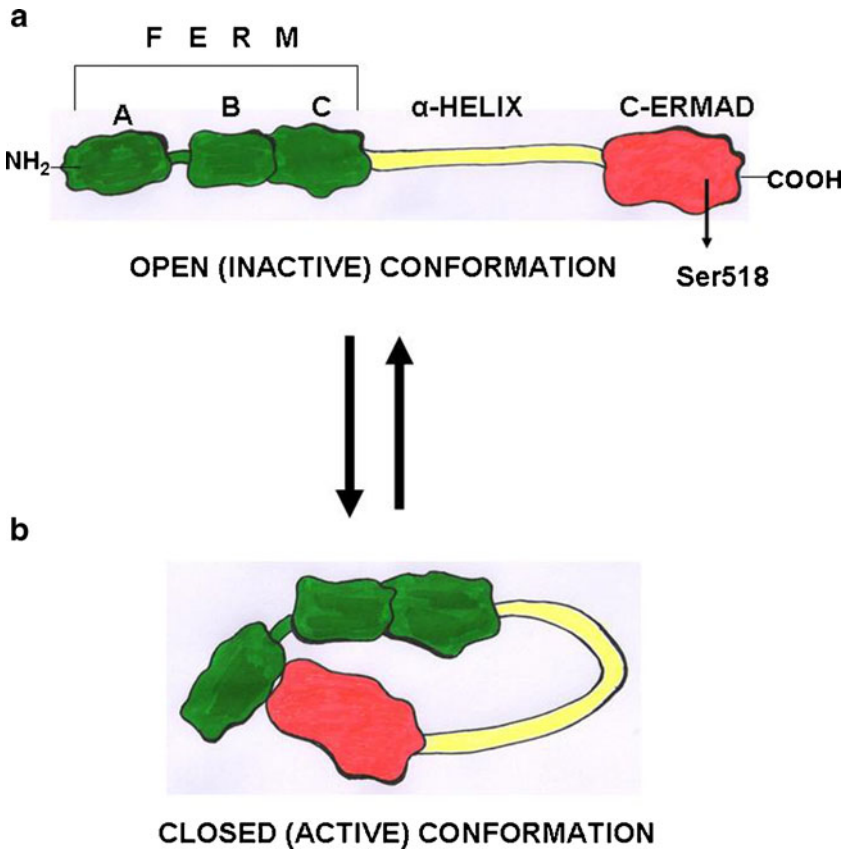

Fig. 1 Schematic illustration of merlin's structure and two conformations, open and closed. **a** The amino terminal, highly conserved, FERM domain consists of A, B and C subdomains. Actin binding site is in the N-terminal domain, more precisely from 178 to 367 residues. Merlin has an alpha-helical region between FERM and the carboxy terminus of the protein. C-ERMAD domain comprising Ser 518 (indicated by an *arrow*) is located at the merlin's carboxy end. **b** Merlin's head-to-tail folding between FERM and C-ERMAD domains renders the protein in closed active conformation upon dephosphorylation of Ser 518

NF2−/− cells develop extraembryonic ectoderm, but have many defects in a variety of other embryonic tissues which indicates NF2 wide ranging role in normal development. As for the NF2+/− mutant mice engineered to lack one copy of the *NF2* gene it has been shown that they are cancer prone and develop a variety of tumors including osteosarcomas, liver tumors, lymphomas, chrondrosarcomas, fibrosarcomas and rhabdomyosarcomas, all of which show a high rate of aggressive behavior and metastasis. Surprisingly, the first generation of NF2+/− mice do not develop the tumor hallmarks of human NF2. It seems that murine schwannomas differ from their human partners, or that the genetic cooperativity leading to NF2 might also be different in two species. Despite this inconsistency, another approach was used to evidence that NF2 loss is responsible—conditional knock out mice model. Schwannomas will develop in conditional knock out mice, the ones in which merlin is specifically knocked out in Schwann cells [17]. Conditional knock out mice were constructed using schwann cell-specific myelin protein zero promoter that controlled Cre recombinase expression and floxed *NF2* genes resulting in P_0-Cre;-$Nf2^{flox/flox}$ mice [18]. Second generation of such mice model most aspects of NF2 and are now used for schwannoma pathogenesis investigations.

NF2 was classified as tumor suppressor by genetic criteria, where loss of heterozygosity studies demonstrate that NF2 patients possess constitutional heterozygous inactivating mutation of one allele of the *NF2* gene, and the tumors they develop harbor somatic mutation of the remaining wild-type allele. Moreover, biallelic inactivation of the *NF2* gene is another genetic event characteristic of all sporadic schwannomas and approximately 60 % of sporadic meningiomas. Another proof that merlin acts as tumor suppressor came from research that showed that overexpression of this protein inhibits both mitogenesis [14, 19] and oncogene-induced transformation [20]. Consequently when re-expressed in human schwannoma cells, merlin managed to reduce survival of schwannoma cells as well as their growth.

Merlin's Interactions and Signaling Roles

The abundance of molecules with which merlin interacts is exceptional. To name a few: CD44, CD43, actin, paxillin, layilin, calpain, betaII-spectrin, RhoGD1, DCC and many others (for extensive reviews see Scoles [21] and Stamenkovic and Yu [4]). Merlin has been shown to interact with cell-surface proteins, proteins involved in cytoskeletal dynamics and proteins involved in regulating ion transport. The main characteristic of cells lacking NF2 protein product is the loss of contact inhibition of proliferation [22, 23]. Associated with loss of contact inhibition, merlin-lacking cells are also known to contain defective adherens junctions [24]. Thus it is understandable that cells that suffered merlin loss show deregulated

adhesion to extracellular matrix which is also shown in schwannoma cells. Another point on merlin's function is that merlin seems to be directly involved in cytoskeletal organization relevant to myelination. Merlin is a linker protein between the plasma membrane and the actin cytoskeleton. It binds actin and tubulin and regulates actin polymerization. His particular role is in the reorganization of cortical cytoskeleton [25].

Recent research on *NF2* gene evidenced that merlin is involved in several different signal transduction pathways [4, 7, 14, 21, 26]. The best characterized are Hippo and Ras/Raf/Mek/Erk pathway. Merlin is a probable regulator of the Hippo also known as the Salvador/Warts/Hippo (SWH) signaling pathway, a pathway that plays important role in tumor suppression by restricting proliferation and promoting apoptosis. The mutations of protein kinase Hippo (Hpo) after which the pathway takes its name, lead to tissue overgrowth, or a hippopotamus-like-phenotype. Merlin and KIBRA are apically-localized proteins and merlin's FERM domain associates with Ex and Kibra forming the Kibra-Ex-Mer (KEM) complex. So merlin acts as an upstream regulator of the core kinase cascade in the Hippo signaling.

At the membrane merlin performs inhibitory actions by regulating growth factor receptors and integrins and thus influencing downstream signaling pathways.

Merlin has been shown to inhibit Ras/Raf/Mek/Erk pathway. Merlin's role in the Ras/Raf/Mek/Erk pathway in normal Schwann cell starts upon the paxilin binding, when merlin forms a complex with erbB2 (HER2) and beta1 integrin at the cell membrane [14, 27]. This complex now with activated merlin being part of it inhibits the Akt (protein kinase B) and MAPK (ERK) signaling by preventing the accumulation of two members of the epidermal growth factor receptor (EGFR) family, erbB2 and erbB3 (HER3). Merlin can also act upstream of Ras/Raf/Mek/Erk pathway by inhibiting Ras and Rac activation after growth factor stimulation [28]. In schwannomas, where merlin is lost the Ras/Raf/Mek/Erk pathway is activated.

Merlin is often enriched in lipid rafts, which are required for receptor internalization and regulation of downstream signaling mediated by those receptors. It is especially involved in receptor tyrosine kinases (RTKs) internalization [29]. Key families of receptor tyrosine kinases are the ErbB family, insulin-like growth factor 1 receptor, platelet-derived growth factor receptor β, and vascular endothelial growth factor receptors.

Merlin inhibits signaling by integrins and RTKs and therefore the activation of downstream pathways, including the Ras/Raf/Mek/Erk, FAK/Src, PI3-kinase/Akt, Rac/PAK/JNK, mTOR (mammalian target of rapamycin) is inhibited too. In line with this is the fact that IGF1 and PDGF are growth factors involved in Schwann cell mitoses [14] and that growth factor receptors (PDGFR, EGFR family) are activated in schwannomas.

In PI3-kinase/Akt signaling merlin directly binds phosphatidylinositols, and this action is mediated by merlin folding conformation and Akt phosphorylation. Merlin suppresses PI3-kinase/Akt signaling through directly binding and inhibiting PIKE-L's stimulatory activity on PI3-kinase. Akt reciprocally inhibits merlin's function by provoking its degradation [30].

Novel reports indicate merlin's connection to yet another important cellular pathway, the wnt signaling pathway [28, 31, 32]. A study by Lau et al. [33] demonstrated the relationship between merlin and wnt signaling in human glioma cells. They showed that merlin's reexpression in human glioma cells decreased the quantity of frizzled-1 (FZD1) receptors (FZD1 binds wnt ligands and activates wnt pathway), and increased the expression of molecules that inhibit wnt signaling, dickkopf-1 (DKK1) and dickkopf-2 (DKK2). So merlin's reexpression reduced wnt signaling. Another paper by Bosco et al. [31] report on significant increase in transcriptionally active nuclear beta-catenin upon merlin deletion. Beta-catenin is the main signaling effector molecule of this pathway and its activation and nuclear transfer starts up the wnt signaling. In this contribution the authors show that when merlin is lost, TCF/LEF/beta-catenin transcription activity increased. Also NF2−/− cells contained an increased active beta-catenin levels, and finally elevations in transcriptional activity of downstream targets of activated beta-catenin were evident in the absence of merlin. A paper by Zhou et al. [32] demonstrated that canonical wnt signaling is activated in primary human schwannoma cells and that activated beta-catenin localizes in the nucleus. Bosco et al. [31] believe that deregulated canonical Wnt signaling is associated with NF2's loss of function. They showed a significant increase in transcriptionally active beta-catenin when merlin protein was deleted. It has been shown previously that cells lacking NF2 loose contact inhibition [4] and are free to proliferate. Associated with loss of contact inhibition, merlin-lacking cells are also known to contain defective adherens junctions [24]. Bosco et al. [31] indicated the relationship of merlin to the wnt as a possible mechanism by which NF2 deficient cells are able to escape contact inhibition. They also showed that elevated nuclear beta-catenin activity in NF2 deficient cells contributes to the growth phenotype of the cells at confluent state. They established the relationship between Rac1 involvement and demonstrate that Rac1 mediated canonical wnt signaling is essential for the loss of contact inhibition in NF2 deficient cells. Another study on high grade human gliomas by Lau et al. [33] discovered an increase in wnt signaling as a result of NF2 loss, specifically the increase of TCF transcription factor activity. To investigate whether wnt signaling is involved in schwannoma Zhou et al. [32] used normal Schwann cells in comparison to Schwanoma cells. They demonstrated that canonical wnt signaling is activated in primary human

schwannoma cells because they found activated beta-catenin localized in the nucleus and wnt target genes c-myc and cyclin D1 overexpressed as a consequence of enhanced transcriptional activities. These findings all collectively describe the influence of merlin on wnt signaling.

In recent studies merlin is characterized as shuttling protein whose involvement in the nucleus is a novel aspect of its functioning. It has been shown that it can migrate into the nucleus, where it inhibits CRL4DCAF1 E3 ubiquitin ligase and consequently suppresses cell growth [25].

Mutational Spectra of *NF2* Gene

In this review I decided to summarize briefly the known mutations of the *NF2* gene. The mutations of the *NF2* gene include splicing defects, frameshifts, nonsense and missense mutations [34–36]. The overall *NF2* mutational spectrum reported by The Human Gene Mutation Database [37] could be categorized as follows: missense/nonsense mutation are very common with reported number of 87, there is 74 splicing mutations, small deletions are the most frequent with reported number of 95, there are 30 small insertions, 44 gross deletions and 6 gross insertions/duplications are reported, additionally there are 3 complex rearrangements. In total there are 345 mutations of the *NF2* gene.

It has also been shown that *NF2* gene has high rate of de novo mutations, so approximately 50 % of NF2 cases harbor de novo mutations for which no other family members can be identified. This suggests a high mutation rate for this gene [5, 17].

Consequence of merlin's mutations depend on the type and their position in the *NF2* gene. For example truncating nonsense mutations or frame-shift mutations that are diagnosed at a younger age will develop severe clinical phenotype with a higher number of schwannomas, while those NF2 patients that harbor large deletions, in-frame indels and missense mutations suffer from much milder disease. Mutations at splice sites cause variations in the severity of NF2 [14, 38, 39].

Moreover, deletions striking exons 15 and 16 at the 3′ end also cause milder clinical features. There are other possible regulatory mechanisms including epigenetic modifications of the regulatory region and posttranscriptional alternative splicing that influence NF2 protein production and clinical phenotype [40].

Clinical Implications

Schwannoma, meningiomas, hamartoma and ependymoma formation within the autosomal dominant neurofibromatosis type 2 (NF2) inherited syndrome are all caused by different mutations in the *NF2* gene. *NF2* is caused by germline mutations in the *NF2* gene on 22q12. The population based birth incidence of NF2 was estimated as 1 case in 33,000–40,000 individuals. The hallmark in this disorder is the presence of bilateral schwannomas involving the eight cranial nerve (vestibular schwannomas) [41, 42].

Ependymomas and hamartomas are relatively rare in comparison to schwannomas and meningiomas. Moreover, only a subset of ependymomas are caused by merlin loss indicating that their overall genetic basis is much more diverse. Therefore those tumors are not going to be discussed in this contribution.

Schwannomas

Schwannomas (neurinomas, neurilemomas) are benign encapsulated tumors of Schwann cells, the main peripheral glia cells, that do not invade the nerve but rather grow around it [43]. It is extremely rare for a schwannoma to transform and become malignant [17, 44]. The majority of schwannomas arise spontaneously and only 4 % are associated with NF2. They represent 8 % of intracranial tumors, and those that strike cerebellopontine angle are in 90 % of cases schwannomas (Fig. 2). In the recent decade a great progress has been made in the molecular and genetic characteristics causative of both sporadic and familial forms of schwannomas. So it is nowadays recognized that alterations of *NF2* gene is a causative event in the tumorigenesis of schwannomas.

Schwannomas also occur spontaneously e. i. sporadically. An annual incidence of sporadic vestibular schwannoma was approximately 1.3 per 100,000 [39]. A population-based study in Denmark showed an estimated incidence of 11.5 cases per million inhabitants per year [45], while the US national tumor registry reported 1.1 cases per 100,000 people per year.

Loss of heterozygosity, *i. e.* gross deletion of *NF2* gene is a common feature found in the majority of sporadic schwannomas. Actually, it is nowadays obvious that all sporadic schwannomas are caused by some kind of alteration of *NF2* gene [36, 46] and the consecutive loss of its protein merlin. The majority of detected deletions and mutations result in a truncated (shorter) protein products [35]. The evidence very strongly suggests that all schwannomas are caused by changes in both gene copies and consequent loss of NF2 protein function [11].

Loss of expression of NF2 protein product merlin is universal finding in all schwannomas examined. The loss of immunoreactivity was reported in many papers [38, 47, 48]. Nevertheless, the intracellular mechanism of the transformation of Schwann cells into schwannomas still needs to be elucidated. It seems that the inactivation of second allele often occurs via a large deletion of the 22q chromosomal region.

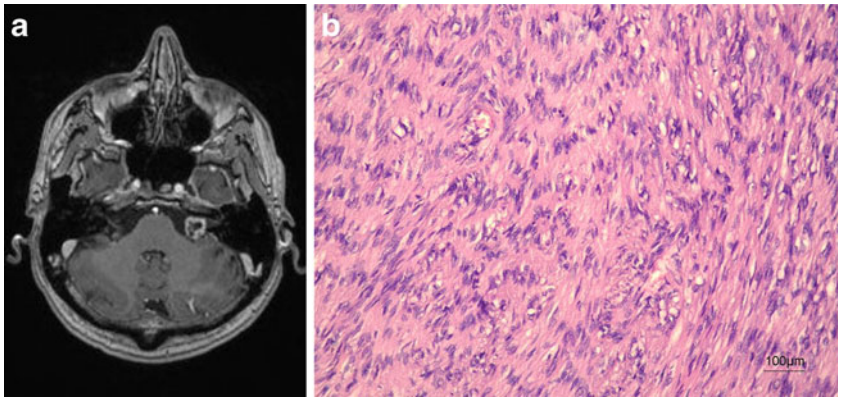

Fig. 2 Vestibular schwannoma. **a** MR image showing lesion in the left pontocerebellar angle. **b** Schwannoma histology, Antoni A. Tumor is composed of compact spindle cells that had twisted nuclei and indistinct cytoplasmic borders (200×, H&E)

Our previous work focuses on the analysis of loss of heterozygosity (LOH) of the *NF2* tumor suppressor gene in a panel of sporadic schwannomas from Croatian patients [49]. Loss of heterozygosity (LOH) of the *NF2* gene, using microsatellite markers, was found in 43.75 % of our schwannoma sample, thus broadening the overall *NF2* mutational spectrum. Our results were in accordance to the frequency of LOHs in sporadic schwannomas reported by other authors [38, 48, 50–54].

In those studies the number of LOHs range from 40 to 80 % depending on the number of genetic markers used and number of cases examined. Bian et al. [50] report on 42.6 % of LOHs. Moreover, they report on the difference in *NF2* LOHs between vestibular and spinal schwannomas and on the association of higher proliferative index to the schwannomas showing LOH. Hadfield et al. [51] report on LOH occurrence in 54 out of 96 (56 %) sporadic vestibular schwannomas. There are reports on higher number of losses by Aarhus et al. [55] who detected 72 % of *NF2* deletions by direct sequencing and Lee et al. [48] who found 77 % of LOHs. Vestibular schwannomas were also analyzed by comparative genomic hybridization in several studies. Loss on 22q was reported in 23 % of sporadic schwannomas examined by Antinheimo et al. [38]. Warren et al. [53] examined 66 sporadic vestibular schwannomas and found 23.7 % of losses; while Koutsimpelas et al. [54] found losses on chromosome 22 in 30 % of their cases. Mantripragada et al. [52] performed a high resolution study using an array covering 1/3 of human chromosome 22 and found LOH in 45 % of schwannomas.

Meningioma

The knowledge on genetic susceptibility for meningioma has once again come from studies of rare genetic syndromes. Besides many other syndromes, meningiomas are a principal feature of neurofibromatosis type 2 (NF2). Loss of expression of NF2 protein product merlin is consistent finding in all NF2 associated meningiomas and in about half

Table 2 NF2 associated tumors and genetic changes

NF2 associated tumors	Genetic changes	Citation
Schwannomas	*NF2* gene mutations, LOH of the 1st *NF2* allele	[5, 9]
	Second mutational hit of the 2nd allele or 2nd allele loss by mitotic recombination or methylation	[11, 14] [17, 35–39] [41, 44] [49–54]
Meningiomas	LOH of *NF2*, inactivating mutations of the 2nd allele	[56–58]
	Loss of other genes residing on chromosome 22	[60] [62] [63] [65]
Schwannomatosis	*INI1/SMARCB1* mutation, loss of a portion of chromosome 22 that contains 2nd *SMARCB1* allele and 1st *NF2* allele, mutation in the 2nd NF2 allele	[51] [67] [68]
Ependymomas	LOH of chromosome 22q	[7]
	Approximately 30 % of ependymomas show merlin loss of expression	[11] [21]

of sporadic cases [56–58]. Meningiomas account for approximately 25 % of primary intracranial and intraspinal neoplasms originating from the meningeal coverings of the brain and the spinal cord with incidence estimated to be 1 per 30,000–40,000 persons in the USA [59]. The morphological features suggest that meningiomas are derived from arachnoidal (meningothelial) cells. The majority of meningiomas corresponds to grade I of WHO classification of CNS tumors and thus are benign, slowly growing tumors [60, 61]. Within the benign category there are several subtypes, including meningothelial, fibrous (fibroblastic), transitional (mixed), psammomatous and angiomatous meningiomas. Yet, these classifications are imprecise with respect to prediction of patient outcome, recurrence or response to treatment. Meningiomas associated with less favorable clinical outcome correspond to grade II (atypical) and those who will exhibit features of malignant behavior—to grade III (anaplastic). The majority of meningiomas suffer losses on 22q including loss of heterozygosity of the *NF2* gene. Up to 60 % of meningiomas carry inactivating mutations in the remaining *NF2* allele. However, many meningiomas are sporadic and of unknown etiology. In approximately 60 % of sporadic meningiomas, the NF2 gene is inactivated by a small mutation, and this is most frequently accompanied by loss of the second allele, usually reflected by loss of the entire chromosome 22 [62–65]. It has been shown that merlin's inactivation is involved in about half of sporadic meningiomas, too. In our previous investigation on sporadic meningiomas two LOHs of the *NF2* gene were found with the D22S929 marker [66].

The remaining percent (40 %) of sporadic meningioma failed to show the aberrations of chromosome 22, nor the mutations in the *NF2* gene. This discrepancy suggests that an alternative pathogenetic mechanism is responsible for the development of these tumors. The gene(s) responsible for the meningiomas with unaffected *NF2* gene remains unknown.

The possibility that other meningioma genes reside on chromosome 22 is also taken into account today, mainly because it has been demonstrated that the frequency of LOH of chromosome 22 exceeds that of *NF2* gene abnormalities. Moreover, investigations on deletions of chromosome 22 have detected losses outside the genetic region of *NF2* gene. A number of investigations have studied specific genes and their role in meningioma, including genes involved in cell cycle regulation, signaling pathways and DNA repair.

Schwannomatosis

Besides being a principal feature of neurofibromatosis type 2 (NF2), schwannomas are also principal in another hereditary tumor disease—Schwannomatosis. Schwannomatosis was recognized as a distinct disorder only recently, and is still very difficult to distinguish schwannomatosis from NF2 or sporadic schwannomas. The main distinction is that it consists of multiple schwannomas without associated vestibular schwannoma typical for NF2 [10, 45]. Most schwannomas in schwannomatosis are also caused by a mutation in the *NF2* gene, nevertheless a novel gene candidate *INI1/SMARCB1* [67], is indicated to be responsible for a part (approximately 20 %) of schwannomatosis-associated schwannomas. This is a tumor suppressor gene located on chromosome 22q11.2 encoding the INI1 protein. This indicates the genetic heterogeneity of schwannomatosis. Additional genetic changes necessary for the development of Schwannomatosis are loss of a portion of chromosome 22 that contains the second SMARCB1 allele and one *NF2* allele whose counterpart would subsequently be inactivated by mutation [68].

NF2 associated tumors are summarized in Table 2.

Conclusion and Future Perspectives

Although many molecular studies have examined genetic alterations of tumor suppressor genes and oncogenes associated with NF2 tumorigenesis, the molecular basis of etiology and pathogenesis of tumors caused by loss of merlin still need to be elucidated.

As merlin tumors are usually benign tumors that respond poorly to classical chemotherapeutics and often result in morbidity with current therapies of choice being surgery and radiosurgery, it is important to develop other novel therapeutic approaches. It is important to understand how merlin's loss causes tumorigenesis which would open the door for new therapies.

The growth factors, RTKs and signaling pathways involved in schwannomas are potential therapeutic objectives for treatment of schwannomas.

Many discoveries have been made on merlin's different functions. However, it is still not clear how those functions are responsible for the development of NF2 tumors. Signaling pathways that are affected by merlin's loss are emerging as possible targets for therapy, but we still need light on their importance priority in schwannoma development. Taking into account many cellular levels of merlin's action it seems that we would need a universal therapeutic molecule.

Acknowledgments This work was supported by grant 108-1081870-1905 from Ministry of Science Sports and Education, Republic of Croatia.

References

1. Rouleau GA, Merel P, Lutchman M, Sanson M, Zucman J, Marineau C, Hoang-Xuan K, Demczuk S, Desmaze C, Plougastel B et al (1993) Alteration in a new gene encoding a putative

membrane-organizing protein causes neurofibromatosis type 2. Nature 363:515–521
2. Trofatter JA, MacCollin MM, Rutter JL, Murrell JR, Duyao MP, Parry DM, Eldridge R, Kley N, Menon AG, Pulaski K et al (1993) A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. Cell 72:791–800
3. Gusella JF, Ramesh V, MacCollin M, Jacoby LB (1999) Merlin: the neurofibromatosis 2 tumor suppressor. Biochim Biophys Acta 1423:M29–M36
4. Stamenkovic I, Yu Q (2010) Merlin, a "magic" linker between the extracellular cues and intracellular signaling pathways that regulate cell motility, proliferation, and survival. Curr Protein Pept Sci 11:471–484
5. Sughrue MI, Yeung AH, Rutkowski MJ, Cheung SW, Parsa AT (2011) Molecular biology of familial and sporadic vestibular schwannomas: implications for novel therapeutics. J Neurosurg 114:359–366
6. Ammoun S, Hanemann CO (2011) Emerging therapeutic targets in schwannomas and other merlin-deficient tumors. Nat Rev Neurol 7:3992–3999
7. Zhou L, Hanemann CO (2012) Merlin, a multi-suppressor from cell membrane to the nucleus. FEBS Lett 586:1403–1408
8. Entrez, the life science search engine (http://www.ncbi.nlm.nih.gov/sites/gquery)
9. McClatchey AI (2007) Neurofibromatosis. Annu Rev Pathol Mech Dis 2:191–216
10. Shimizu T, Seto A, Maita N, Hamada K, Tsukita S, Hakoshima T (2002) Structural basis for neurofibromatosis type 2. Crystal structure of the merlin FERM domain. J Biol Chem 277:10332–10336
11. Hanemann CO (2008) Magic but treatable? Tumours due to loss of Merlin. Brain 131:606–615
12. UniProtKB/Swiss-Prot (http://www.uniprot.org/uniprot/P35240#section_comments)
13. Laulajainen M, Melikova M, Muranen T, Carpen O, Gronholm M (2012) Dictinct overlapping sequences at the carboxy-terminus of merlin regulate its tumor suppressor and morphogenic activity. J Cell Mol Med 16:2161–2175
14. Carroll SL (2012) Molecular mechanisms promoting the pathogenesis of Schwann cell neoplasms. Acta Neuropathol 123:321–348
15. Alfthan K, Heiska L, Grönholm M, Renkema GH, Carpén O (2004) Cyclic AMP-demendent protein kinase phosphorylates merlin at serine 518 independently of p21 activated kinase and promotes merlin-ezrin heterodimerization. J Biol Chem 279:18559–18566
16. McClatchey AI, Saotome I, Ramesh V, Gusella JF, Jacks T (1997) The NF2 tumor suppressor gene product is essential for extraembryonic development immediately prior to gastrulation. Genes Dev 11:1253–1265
17. Hanemann CO, Evans DG (2006) News on the genetics, epidemiology, medical care and translational research of Schwannomas. J Neurol 253:1533–1541
18. Giovannini M, Robanus-Maandag E, van der Valk M, Niwa-Kawakita M, Abramowski V, Goutebroze L, Woodruff JM, Berns A, Thomas G (2000) Conditional biallelic NF2 mutation in the mouse promotes manifestations of human neurofibromatosis type 2. Genes Dev 14:1617–1630
19. Lutchman M, Rouleau GA (1995) The neurofibromatosis type 2 gene product, schwannomin, suppresses growth of NIH 3T3 cells. Cancer Res 55:2270–2274
20. Tikoo A, Varga M, Ramesh V, Gusella J, Maruta H (1994) An anti-Ras function of neurofibromatosis type 2 gene product (NF2/Merlin). J Biol Chem 269:23387–23390
21. Scoles DR (2008) The merlin interacting proteins reveal multiple targets for NF2 therapy. Biochim Biophys Acta 1785:32–54
22. Morrison H, Sherman LS, Legg J, Banine F, Isacke C, Haipek CA, Gutmann DH, Ponta H, Herrlich P (2001) The NF2 tumor suppressor gene product, merlin, mediates contact inhibition of growth through interactions with CD44. Genes Dev 15:968–980
23. Okada T, Lopez-Lago M, Giancotti FG (2005) Merlin/NF-2 mediates contact inhibition of growth by suppressing recruitment of Rac to the plasma membrane. J Cell Biol 171:361–371
24. Lallemand D, Curto M, Saotome I, Giovannini M, McClatchey AI (2003) NF2 deficiency promotes tumorigenesis and metastasis by destabilizing adherens junctions. Genes Dev 17:1090–1100
25. Li W, Cooper J, Karajannis MA, Giancotti FG (2012) Merlin: a tumour suppressor with functions at the cell cortex and in the nucleus. EMBO Rep 13:204–215
26. Kim H, Kwak NJ, Lee JY, Choi BH, Lim Y, Ko YJ, Kim YH, Huh PW, Lee KH, Rha HK, Wang YP (2004) Merlin neutralizes the inhibitory effect of Mdm2 on p53. J Biol Chem 279:7812–7818
27. Fernandez-Valle C, Tang Y, Ricard J, Rodenas-Ruano A, Taylor A, Hackler E, Biggerstaff J, Iacovelli J (2002) Paxillin binds schwannomin and regulates its density-dependent localization and effect on cell morphology. Nat Genet 31:354–362
28. Morrison H, Sperka T, Manent J, Giovannini M, Ponta H, Herrlich P (2007) Merlin/neurofibromatosis type 2 suppresses growth by inhibiting the activation of Ras and Rac. Cancer Res 67:520–527
29. Fraenzer JT, Pan H, Minimo L Jr, Smith GM, Knauer D, Hung G (2003) Overexpression of the NF2 gene inhibits schwannoma cell proliferation through promoting PDGFR degradation. Int J Oncol 23:1493–1500
30. Okada M, Wang Y, Jang SW, Tang X, Neri LM, Ye K (2009) Akt phosphorylation of merlin enhances its binding to phosphatidylinositols and inhibits the tumor-suppressive activities of merlin. Cancer Res 69:4043–4051
31. Bosco EE, Nakai Y, Hennigan RF, Ratner N, Zheng Y (2010) *NF2*-deficient cells depend on the Rac1-canonical Wnt signaling pathway to promote the loss of contact inhibition of proliferation. Oncogene 29:2540–2549
32. Zhou L, Ercolano E, Ammoun S, Schmid MC, Barczyk MA, Hanemann CO (2011) Merlin-deficient human tumors show loss of contact inhibition and activation of Wnt/β-Catenin signaling linked to the PDGFR/Src and Rac/PAK pathways. Neoplasia 13:1101–1112
33. Lau YKI, Murray LB, Houshmandi SS, Nu Y, Gutmann DH, Yu Q (2008) Merlin is a potent inhibitor of glioma growth. Cancer Res 68:5733–5742
34. Evans DG, Huson SM, Donnai D, Neary W, Blair V, Teare D, Newton V, Strachan T, Ramsden R, Harris R (1992) A genetic study of type 2 neurofibromatosis in the United Kingdom. I. Prevalence, mutation rate, fitness, and confirmation of maternal transmission effect on severity. J Med Genet 29:841–846
35. Jacoby LB, MacCollin M, Barone R, Ramesh V, Gusella JF (1996) Frequency and distribution of NF2 mutations in schwannomas. Genes Chromosom Cancer 17:45–55
36. Irving RM, Harada T, Moffat DA, Hardy DG, Whittaker JL, Xuereb JH, Maher ER (1997) Somatic neurofibromatosis type 2 gene mutations and growth characteristics in vestibular schwannoma. Am J Otol 18:754–760
37. The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=NF2)
38. Antinheimo J, Sallinen SL, Sallinen P, Haapasalo H, Helin H, Horelli-Kuitunen N, Wessman M, Sainio M, Jääskeläinen J, Carpén O (2000) Genetic aberrations in sporadic and neurofibromatosis 2 (NF2)-associated Schwannomas studied by comparative genomic hybridization (CGH). Acta Neurochir 142:1099–1105
39. Ahronowitz I, Xin W, Kiely R, Sims K, MacCollin M, Nunes FP (2007) Mutational spectrum of NF2 gene: a meta-analysis of 12 years of research and diagnostic laboratory findings. Hum Mutat 28:1–12
40. Welling DB, Akhmametyeva EM, Daniels RL, Lasak JM, Zhu L, Miles-Markley BA, Chang LS (2000) Analysis of the human

neurofibromatosis type 2 gene promoter and its expression. Otolaryngology 123:413–418
41. Gutmann DH, Geist RT, Xu H, Kim JS, Saporito-Irwin S (1998) Defects in neurofibromatosis 2 protein function can arise at multiple levels. Hum Mol Genet 7:335–345
42. Fong B, Barkhoudarian G, Pezeshkian P, Parsa AT, Gopen Q, Yang I (2011) The molecular biology and novel treatments of vestibular schwannomas. J Neurosurg 115:906–914
43. Scheithauer BW, Louis DN, Hunter S, Woodruff JM, Antonescu CR (2007) Tumours of the cranial and paraspinal nerves. Schwannoma. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds) WHO classification of tumours of the central nervous system, 4th edn. International Agency for Research on Cancer, Lyon, pp 152–155
44. Ghosh A, Talwar OP, Pradhan SV (2010) Tumour and tumour-like conditions of peripheral nerve origin: ten years' experience. Kathmandu Univ Med J 29:97–101
45. Celis-Aguilar E, Lassaletta L, Torres-Martin M, Rodrigues FY, Nistal M, Castresana JS, Gavilan J, Ray JA (2012) The molecular biology of vestibular schwannomas and its association with hearing loss: a review. Genet Res Int. Article ID 856157:10. doi:10.1155/2012/856157
46. Irving RM, Moffat DA, Hardy DG, Barton DE, Xuereb JH, Maher ER (1993) Molecular genetic analysis of the mechanism of tumorigenesis in acoustic neuroma. Arch Otolaryngol Head Neck Surg 119:1222–1228
47. Hitotsumatsu T, Iwaki T, Kitamoto T, Mizoguchi M, Suzuki SO, Hamada Y, Fukui M, Tateishi J (1997) Expression of neurofibromatosis 2 protein in human brain tumors: an immunohistochemical study. Acta Neuropathol 93:225–232
48. Lee DJ, Maseyesva B, Westra W, Long D, Niparko JK, Califano J (2006) Microsatellite analysis of recurrent vestibular Schwannoma (acoustic neuroma) following stereotactic radiosurgery. Otol Neurotol 27:213–219
49. Pećina-Šlaus N, Zeljko M, Pećina HI, Nikuševa Martić T, Bačić N, Tomas D, Hrašćan R (2012) Frequency of loss of heterozygosity of the NF2 gene in schwannomas from Croatian patients. Croat Med J 53:321–327
50. Bian LG, Sun QF, Tirakotai W, Zhao WG, Shen JK, Luo QZ, Bertalanffy H (2005) Loss of heterozygosity on chromosome 22 in sporadic schwannoma and its relation to the proliferation of tumor cells. Chin Med J (Engl) 118:1517–1524
51. Hadfield KD, Smith MJ, Urquhart JE, Wallace AJ, Bowers NL, King AT, Rutherford SA, Trump D, Newman WG, Evans DG (2010) Rates of loss of heterozygosity and mitotic recombination in NF2 schwannomas, sporadic vestibular schwannomas and schwannomatosis schwannomas. Oncogene 29:6216–6221
52. Mantripragada KK, Buckley PG, Benetkiewicz M, De Bustos C, Hirvela C, Jarbo C, Bruder CE, Wensman H, Mathiesen T, Nyberg G, Papi L, Collins VP, Ichimura K, Evans G, Dumanski JP (2003) High-resolution profiling of an 11 Mb segment of human chromosome 22 in sporadic schwannoma using array-CGH. Int J Oncol 22:615–622
53. Warren C, James LA, Ramsden RT, Wallace A, Baser ME, Varley JM, Evans DG (2003) Identification of recurrent regions of chromosome loss and gain in vestibular schwannomas using comparative genomic hybridization. J Med Genet 40:802–806
54. Koutsimpelas D, Felmeden U, Mann WJ, Brieger J (2011) Analysis of cytogenetic aberrations in sporadic vestibular schwannoma by comparative genomic hybridization. J Neurooncol 103:437–443
55. Aarhus M, Bruland O, Sætran HA, Mork SJ, Lund-Johansen M, Knappskog PM (2010) Global gene expression profiling and tissue microarray reveal novel candidate genes and down-regulation of the tumor suppressor gene CAV1 in sporadic vestibular schwannomas. Neurosurgery 67:998–1019
56. Ueki K, Wen-Bin C, Narita Y, Asai A, Kirino T (1999) Tight association of loss of merlin expression with loss of heterozygosity at chromosome 22q in sporadic meningiomas. Cancer Res 59:5995–5998
57. Kros J, de Greve K, van Tilborg A, Hop W, Pieterman H, Avezaat C, Lekane dit Deprez R, Zwarthoff E (2001) NF2 status of meningiomas is associated with tumour localization and histology. J Pathol 194:367–372
58. Fuller CE, Perry A (2005) Molecular diagnostics in central nervous system tumors. Adv Anat Pathol 12:180–194
59. Claus EB, Bondy ML, Wiemels JL, Wrensch M, Black PM (2005) Epidemiology of intracranial meningioma. Neurosurgery 57:1088–1094
60. Riemenschneider MJ, Perry A, Reifenberger G (2006) Histological classification and molecular genetics of meningiomas. Lancet Neurol 5:1045–1054
61. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P (2007) The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 114:97–109
62. Lee JY, Finkelstein S, Hamilton RL, Rekha R, King JT Jr, Omalu B (2004) Loss of heterozygosity analysis of benign, atypical, and anaplastic meningiomas. Neurosurgery 55:1163–1173
63. Ragel BT, Jensen RL (2005) Molecular genetics of meningiomas. Neurosurg Focus 119:E9
64. Barnholtz-Sloan JS, Kruchko C (2007) Meningiomas: causes and risk factors. Neurosurg Focus 23:E2
65. Simon M, Boström JP, Hartmann C (2007) Molecular genetics of meningiomas: from basic research to potential clinical applications. Neurosurgery 60:787–798
66. Pećina-Šlaus N, Nikuševa Martić T, Deak AJ, Zeljko M, Hrašćan R (2010) Genetic and protein changes of E-cadherin in meningiomas. J Cancer Res Clin Oncol 136:695–702
67. Hulsebos TJ, Plomp AS, Wolterman RA, Robanus-Maandag EC, Baas F, Wesseling P (2007) Germline mutation of INI1/SMARCB1 in familial Schwannomatosis. Am J Hum Genet 80:805–810
68. Plotkin SR, Blakeley JO, Evans DG, Hanemann CO, Hulsebos TJ, Hunter-Schaedle K, Kalpana GV, Korf B, Messiaen L, Papi L, Ratner N, Sherman LS, Smith MJ, Stemmer-Rachamimov AO, Vitte J, Giovannini M (2013) Update from the 2011 International Schwannomatosis Workshop: from genetics to diagnostic criteria. Am J Med Genet 161:405–416