Tumor biological characteristics of Vestibular Schwannoma

Vries, M. de

CHAPTER 2

Tumor Biology of Vestibular Schwannoma: A Review of Experimental Data on the Determinants of Tumor Genesis and Growth Characteristics

Maurits de Vries, Andel G. L. van der Mey and Pancras C. W. Hogendoorn

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Abstract

Objective: provide an overview of the literature on vestibular schwannoma biology with special attention to tumor behavior and targeted therapy.

Background: vestibular schwannomas are benign tumors originating from the eighth cranial nerve and arise due to inactivation of the *NF2* gene and its product merlin. Unraveling the biology of these tumors helps to clarify their growth pattern and is essential in identifying therapeutic targets.

Methods: PubMed search for English language articles on vestibular schwannoma biology from 1994 till 2014.

Results: activation of merlin and its role in cell signaling seem key aspects of vestibular schwannoma biology. Merlin is regulated by proteins like CD44, Rac and myosin phosphatase targeting subunit 1 (MYPT1). The tumor suppressive functions of merlin are related to receptor tyrosine kinases, such as the platelet-derived growth factor receptor (PDGFR) and vascular endothelial growth factor receptor (VEGF). Merlin mediates the Hippo pathway and acts within the nucleus by binding E3 ubiquiting ligase $CRL4^{DCAF1}$. Angiogenesis is an important mechanism responsible for the progression of these tumors and is affected by processes like hypoxia and inflammation. Inhibiting angiogenesis by targeting VEGF seems to be the most successful pharmacological strategy but additional therapeutic options are emerging.

Conclusion: over the years the knowledge on vestibular schwannoma biology has significantly increased. Future research should focus on identifying new therapeutic targets by investigating vestibular schwannoma (epi)genetics, merlin function and tumor behavior. Besides identifying novel targets, testing new combinations of existing treatment strategies can further improve vestibular schwannoma therapy.

Introduction

Sporadic vestibular schwannomas (VS) are benign tumors recapitulating the differentiation repertoire of the myelin-forming Schwann cells of the vestibular branch of the eighth cranial nerve. Vestibular schwannomas derive within the internal auditory canal, often extending into the cerebellopontine angle. Associated symptoms are hearing loss, tinnitus and vertigo. Large tumors can cause paralysis of adjacent cranial nerves and brainstem compression. The majority of vestibular schwannomas occur as unilateral sporadic tumors(>90%)[1]. Bilateral tumors are pathognomonic for the hereditary disorder neurofibrosis type 2 (NF2). In this review we discuss both but mainly focus on the sporadic tumors. Recent years the incidence of vestibular schwannomas has increased to approximately 20 per million people per year [2-4]. This is probably a consequence of the increased application of magnetic resonance imaging (MRI) scanning resulting in the identification of more subclinical cases. Therapeutic management of vestibular schwannomas comprises three strategies i.e. microsurgery, radiotherapy or serial radiological observation. So far pharmacological treatment options are scarce [5-7]. An important aspect determining the most suitable therapy is growth rate. Some tumors remain stable for years while others grow relatively fast (Figure 1.) The biological background of this phenotypical heterogeneity is largely unknown. This review provides an overview of the literature on vestibular schwannoma biology with special attention to tumor behaviour and targeted therapy.

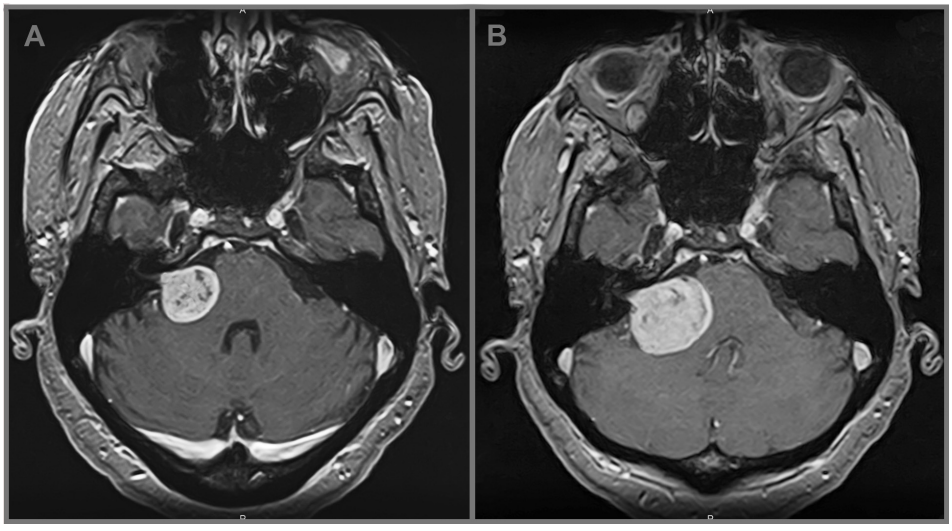

Figure 1. Sequential T1-weighted gadolinium enhanced magnetic resonance imaging scans of a fast-growing sporadic VS. This tumor more than doubled in volume from 4.25 ml (A) to 11.75 ml (B) in less than 10 months causing compression of the brainstem.

NF2 gene

An essential contribution to the understanding of vestibular schwannoma biology was the isolation of the neurofibromatosis type-2 gene (*NF2)* [8,9]. *NF2* encodes for the tumor suppressor protein merlin. This gene is located on chromosome 22q12 and contains 17 exons. Loss of functional merlin is essential in schwannoma pathogenesis [10]. Heterozygous germline inactivating mutations affecting *NF2* cause the autosomal dominant disorder neurofibrosis type-2 and biallelic somatic mutations of *NF2* are found in sporadic vestibular schwannomas [11].

Merlin Structure and Activation

Merlin is a cytoskeletal protein encoded by the *NF2* gene. It shows similarity to the ERM proteins, ezrin, radixin and moesin. These proteins play an important role in linking the actin cytoskeleton with plasma membranes [12]. “Merlin” is an acronym for “Moesin-Ezrin-Radixin-like Protein”. Merlin consists of an relatively conserved N-terminal FERM (Four-point-one, ezrin, radixin, moesin) domain followed by a coil-coil domain and a Carboxyl-terminal domain [13] (Figure 2).

The FERM domain is a membrane-binding module resembling the domain of the ERM proteins except for the C-terminal domain which lacks an actin-binding motif [14-16]. Instead, merlin directly binds actin with residues in the glutathione S-transferase N-terminal domain [17] or indirectly in association with II-spectrin or fodrin [18].
Merlin exists in an open and closed state. Dephosphorylation of merlin causes the protein to close. The closed conformation of merlin is the active tumor suppressor [19-21]. Promitogenic signals initiated by membrane-bound integrins and receptor tyrosine kinases are transduced by the signalling protein Rac which in turn activates p21-activated kinase (PAK) [22,23]. Activated PAK phosphorylates merlin at amino acid serine 518 [19,24,25]. This phosphorylation induces an open conformation of merlin, thereby inhibiting its tumor suppressor function. In addition to PAK merlin is also phosphorylated by protein kinase A (PKA)[26]. PKA mediated phosphorylation not only takes place at serine 518 but serine 10 as well [27]. The effect of phosphorylation at serine 10 is not entirely clear but it is suggested to induce changes of the actin cytoskeleton.
Conversely to Rac, engagement of cadherins or loss of mitogenic signalling inactivates PAK, leading to increased levels of closed, activated merlin [22]. In accordance with these observations, inactivation of merlin leads to loss of contact inhibition [28,29] and accelerated progression of the cell cycle [30]. By combining signals from cadherins and integrins merlin mediates cell cycle progression.
Another regulator of merlin is CD44, a transmembrane hyaluronic acid receptor involved in cell adhesion, matrix adhesion and cell migration [31]. Merlin mediates contact inhibition dependent cell growth by its interaction with CD44. Through these interactions merlin and

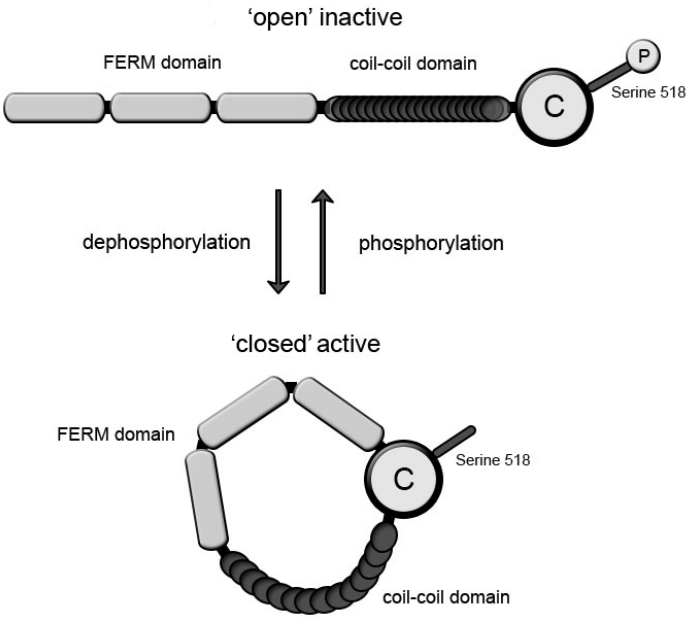

Figure 2. Merlin structure. Merlin has three structural sections: the N-terminal FERM domain followed by a coil-coil domain and a Carboxyl-terminal domain. Dephosphorylation of merlin at amino acid Serine 518 causes the protein to fold and become active

CD44 may function as a switch controlling cell growth arrest or proliferation [32,33]. Merlin can also be activated by myosin phosphatase targeting subunit 1 (MYPT1). This protein dephosphorylates merlin at amino acid serine 518 [20,34]. The concept of MYPT1 mediated activation of merlin is supported by the observation that CPI-17 (protein kinase C-potentiated phosphatase inhibitor of 17 kDa), a cellular inhibitor of MYPT1, causes loss of function of merlin [34]. Figure 3 provides an overview of various interactions involved in merlin regulation.

Merlin's role in cell signalling

Contact mediated inhibition is an important mechanism regulating cell growth. The tumor suppressive role of merlin seems largely affect by contact inhibition [23,32,35,36]. Identifying cellular pathways in which merlin participates may provide targets for treatment. Examples of targeted therapy are tyrosine kinase inhibitors, which have been successfully employed for solid neoplasms like gastrointestinal stromal tumors as well as leukemia's[37,38]. Tyrosine kinases are enzymes involved in the activation of numerous cell signalling cascades, when inhibited they can slow down or arrest tumor progression. It is because of developments like these that a significant proportion of schwannoma related research is now devoted to clarifying the function of merlin and the cell signalling pathways it affects. To maintain comprehensibility only the most well-established interactions will be discussed.

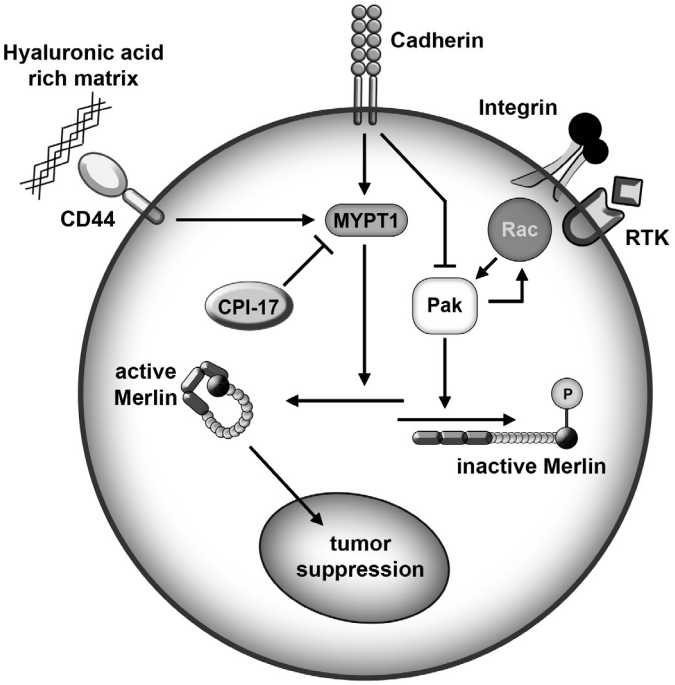

Figure. 3. Merlin activation. Cell-to-cell adhesions and CD44 activate MYPT1, which dephosphorylates merlin resulting in a closed and active protein conformation. Conversely, integrins and receptor tyrosine kinases activate Pak, which phosphorylates merlin, inducing an open and inactivated confirmation.

Merlin's tumor suppressor function is linked to the integrin mediated Rac pathway, which is involved in actin remodelling, cell cycle control, transcription and apoptosis. Shaw et al. [19] were the first to describe this association. They demonstrated merlin's ability to negatively regulate Rac, this was confirmed by additional studies [23,39]. A downstream target of Rac is PAK, the kinase responsible for the activation of merlin. The interaction between merlin, Rac and PAK suggests a positive feedback loop between merlin and PAK.

Merlin has been proposed to suppress proliferation by inhibiting receptor tyrosine kinases (RTKs) including the ErbB receptors, the platelet-derived growth factor receptor (PDGFR), the insulin-like growth factor 1 receptor (IGF1R) and the vascular endothelial growth factor (VEGF) receptor [28,35,40-42]. This is confirmed by the fact that proteins of the oncogenic Ras/Raf/MEK/ERK and PI3K/AKT pathways, which are downstream of these RTKs, are strongly activated in merlin deficient schwannoma cell models [43,44].
Merlin seems to act as a negative regulator of the mammalian target of rapamycin complex 1 (mTORC1), a kinase complex that regulates cell growth, cell proliferation, cell motility and cell survival [45]. mTORC1 seems activated in merlin deficient meningioma cells [46]. This is

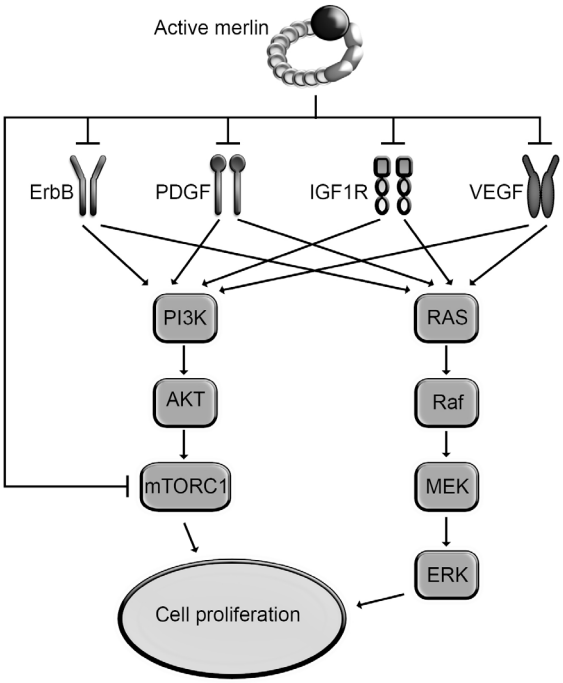

Figure 4. Merlin signalling. Merlin is proposed to suppress proliferation by inhibiting several receptor tyrosine kinases and their downstream signalling pathways.

supported by the correlation between loss of merlin and mTORC1 activation observed in mesothelioma cell lines [30]. The significance of mTORC1 as an effector of merlin is emphasized by the fact that the mTOR kinase inhibitor, Torin 1, could successfully block mTORC1 and AKT in merlin deficient meningioma cells leading to inhibited cell proliferation[47]. See figure 4 for an overview of kinases affected by merlin.

Merlin also mediates the Hippo pathway. This pathway controls organ size through regulation of cell proliferation and apoptosis. Mutations affecting this pathway are associated with different types of cancer [48]. A study investigating Hippo signalling in Drosophila showed that merlin is required for cell proliferation arrest and apoptosis [49]. This observation is supported by studies on meningioma cell lines and primary meningioma tumors which demonstrated that merlin suppresses the oncoprotein YAP (Yes-associated protein), a member of the Hippo pathway capable of stimulating cell proliferation [50,51].

Most of merlin's interactions take place around the plasma membrane. A recent study showed that merlin also accumulates in the nucleus where it binds the E3 ubiquitin ligase $CRL4^{DCAF1}$ blocking its activity [52-54]. $CRL4^{DCAF1}$ has been implicated to induce an elaborate oncogenic program of gene expression [55]. Interactions between merlin and $CRL4^{DCAF1}$ seem to be essential for tumor suppression by merlin.

NF2 mutations in sporadic VS

To date, the genetic profile of vestibular schwannomas has not been fully characterized. The only consistent genetic alteration is inactivation of the *NF2* gene. Multiple mutation analyses screening for *NF2* mutations in sporadic vestibular schwannomas have been described. Reports on the number of tumors containing a proven *NF2* mutation range from 15 to 84% (table 1). Most mutations are small deletions and point mutations [56-61], resulting in truncated proteins. A significant proportion of vestibular schwannomas did not harbour a proven *NF2* mutation. Studies investigating the *NF2* gene product, both at RNA- as well as protein level, demonstrated decreased expression of *NF2* gene products in a much higher percentage of tumors then expected with regard to the percentage of tumors containing an *NF2* mutation [10,62-65]. This difference could be explained by the involvement of other (epi)genetic changes that cause down-regulation of *NF2* expression. Another reason could be the fact that the utilized mutation detection methods were not sensitive enough. A possible factor impairing the sensitivity of these analyses is contamination of tumor tissue with for instance tumor invading cells of the intratumoral infiltrate. This theory is supported by our findings regarding the presence of tumor infiltrating macrophages in vestibular schwannomas [66,67].

Epigenetic alterations are involved in the development of many tumors [71,72]. Hypermethylation of CpG Islands in the promoter region leading to gene silencing is an important epigenetic mechanism causing tumor suppressor inactivation [73]. Aberrant methylation of *NF2* has been investigated in several studies. Kino et. al.[74] analysed 23 vestibular schwannomas and demonstrated aberrant methylation of *NF2* in 14 tumors, suggesting it as an alternative pathway of *NF2* inactivation. Gonzalez-Gomez [75] et. al. reported hypermethylation of *NF2* in just 6 out of 31 sporadic schwannomas. An even lower percentage was reported by Kullar et al.[68]. They found aberrant methylation of *NF2* in 4 out of 40 sporadic vestibular schwannomas. Finally Lee et. al.[69] investigated 30 vestibular schwannomas and found no aberrant methylation at all. The results of these studies vary considerably and do not provide a sufficient explanation for the subpopulation of vestibular schwannomas lacking a proven *NF2* mutation.

Global gene expression profiling of sporadic vestibular schwannomas

Global gene expression profiling experiments provide powerful methods to analyse the expression pattern of a large panel of genes. Welling et al. [76] were one of the first to perform a cDNA microarray analysis on vestibular schwannomas. They studied 7 tumors and identified several deregulated genes. Among the up-regulated genes were osteonectin (SPARC), an angiogenesis mediator, and RhoB GTPase, which is important in cell signaling. Among the down-regulated genes was LUCA-15 which is related to apoptosis. Ezrin, a relative of merlin, was also down-regulated in a majority of tumors. This studies' main limitation was the small number of samples, making statistical analysis difficult.

Table 1. NF2 mutations in sporadic vestibular schwannomas

Author (ref.nr.)	Year	NF2 mutation rate
Irving et al. [58]	1994	13 out of 85 (15%)
Sainz et al.[10]	1994	17 out of 26 (65%)
Welling et al. [56]	1996	19 out of 29 (66%)
Jacoby et al. [57]	1996	41 out of 49 (84%)
Hadfield et al. [61]	2010	65 out of 98 (66%)
Aarhus et al. [59]	2010	19 out of 25 (76%)
Kullar et al. [68]	2010	12 out of 40 (30%)
Lee et al. [69]	2012	16 out of 30 (53%)
Lassaletta et al. [60]	2013	25 out of 51 (49%)
Zhang et al.[65]	2013	50 out of 145 (35%)
Torres-Martin [70]	2013	23 out of 31 (74%)

Epigenetic alterations of *NF2*

A microarray analysis by Cayé-Thomasen et al. [77] investigated 16 vestibular schwannomas and compared their gene expression pattern with 3 vestibular nerves. An interesting up-regulated gene was platelet-derived growth factor D which is involved in cell cycle regulation. PTEN (phosphatase and tensin homolog deleted on chromosome 10), a tumor suppressor gene and major regulator of the PI3K/AKT pathway, was also up-regulated. The authors suggested PTEN up-regulation as compensatory for the lack of merlin inhibition. Comparison of the results of Cayé-Thomasen et al. and Welling et al. revealed an association related to SPARC. Welling et al found this gene to be up-regulated while the scavenger receptor stabilin-1, involved in SPARC degradation, was up-regulated in the analysis by Cayé-Thomasen et al. Subsequently Aarhus et al. [59] demonstrated up-regulation of SPARC as well, emphasizing the role of this gene in vestibular schwannoma biology.

Another finding of Aarhus et al. was the down-regulation of tumor suppressor gene CAV1, suggesting that loss of CAV1 participates in vestibular schwannoma formation. Additionally they performed a network and pathway analysis which indicated the ERK pathway as the central core linking the differentially expressed genes.

Coinciding results were reported in a microarray analysis by Torres-Martin et al. [70] They postulated that down regulation of CAV1 in schwannomas leads to deregulation of MET, a tyrosine kinase receptor involved in cellular mechanisms like proliferation, motility and migration. Table 2 provides an overview of these data.

An important consideration when interpreting the results of these expression profiling studies is the issue of the control tissue. Most studies used peripheral nerve tissue. Peripheral nerves predominantly contain axons surrounded by Schwann cells whereas tumor tissue mainly consists of schwannoma cells. This proportional discrepancy in tissue type can cause non-tumor-related differential cDNA expression which may obscure the actual results.

Table 2. global gene expression

Series (refnr.)	Gene	Function	Regulation status
Welling et.al. [76]	SPARC	angiogenesis	up-regulated
	RhoB GTPase	promotion of cellular functions related to cancerous cells	up-regulated
	LUCA-15	apoptosis	down-regulated
Cayé-Thomassen et.al [77]	PDGFD	cell growth and division	up-regulated
	PTEN	tumor supressor	up-regulated
	Stabilin-1	degradation of SPARC	up-regulated
Aarhus et.al. [59]	SPARC	angiongenesis	up-regulated
	CAV1	tumor supressor	down-regulated
Torres-Martin et.al. [70]	CAV1	tumor supressor	down-regulated

Angiogenesis and the role of the tumor microenvironment

Although vestibular schwannomas are relatively slow-growing neoplasms they still require angiogenesis to progress beyond a certain size [78]. Multiple angiogenesis stimulating factors have been identified; the best established is vascular endothelial growth factor (VEGF). VEGF is expressed by vestibular schwannoma cells [79-82] and several studies have correlated the degree of VEGF expression with clinical parameters such as tumor growth [79,81], tumor volume [83] and microvessel-density [83]. VEGF expression can be induced by hypoxia in response to the production of HIF-1alpha (Hypoxia inducible factor 1alpha) [84,85]. Diensthuber et al. [86] studied HIF-1alpha in sporadic vestibular schwannomas and demonstrated a relation between HIF-1alpha expression and cell proliferation. Next to hypoxia there are other microenvironmental factors regulating angiogenesis and tumor progression. Moller et. al. investigated matrix metalloproteinase-9 (MMP-9), an enzyme involved in migration and invasion of endothelial cells during angiogenesis. They studied 37 sporadic vestibular schwannomas and demonstrated a correlation between MMP-9 expression and tumor growth [87]. Inflammation is also capable of influencing tumor behavior. Macrophages form the major determinants of intratumoral inflammation. These so called tumor associated macrophages are associated with angiogenesis, cell growth and down-regulation of the immune response [88]. We performed a study on 68 sporadic [89]vestibular schwannomas and found a correlation between the expression of CD68 positive macrophages, tumor size and angiogenesis [66]. In a subsequent study we were able to support the concept of inflammation mediated tumor progression by linking macrophage expression to tumor growth [67]. The active role of inflammation in vestibular schwannomas is denoted by the presence of the enzyme COX-2. [90] COX-2 is expressed at sites of inflammation and effects angiogenesis [91]. Vestibular schwannomas with higher proliferation rates show higher COX-2 expression[90]. Vestibular schwannoma angiogenesis also seems to be stimulated by the down-regulation of the antiangiogenic factor semaphoring 3F (SEMA3F)[7]. A study by Wong et al.[92]

demonstrated the ability of merlin to up-regulate SEMA3F through Rac1 thereby decreasing angiogenesis. All together these studies support the importance of angiogenesis in vestibular schwannoma development, making it an interesting target for pharmacotherapeutic treatment.

Targeted therapy

As mentioned in previous paragraphs the increasing biological knowledge on vestibular schwannomas helps to identify targets for therapy. Next to angiogenesis other targets are emerging. Various components of the cell signalling pathways affected by merlin, like the receptor tyrosine kinases IGF1R, EGFR and PDGF, might also form targets for therapy. These growth factors are normally supressed by merlin but can be inhibited pharmacologically as well.

Current research on targeted therapy for vestibular schwannomas primarily focuses on the neurofibrosis type 2 related tumors. This paragraph discusses the latest developments regarding these pharmacotherapeutic options.

The angiogenesis inhibiting drug bevacizumab is an anti-VEGF antibody approved by the U.S. Food and Drug Administration for the treatment of several types of cancer. Plotkin et al. [7] were the first to investigate the effect of bevacizumab in NF2 patients. They demonstrated tumor shrinkage and mild hearing improvement in 9 out of 10 subjects. Mautner et al. [5] reported similar results. The effect of anti-VEGF therapy was also confirmed by Wong et al. [6]. They showed that angiogenesis inhibitors bevacizumab or vandetanib decreased vascularisation and growth rate of schwannoma xenografts in mice. Finally, a retrospective study on 31 NF2 patients demonstrated hearing improvement and tumor shrinkage with bevacizumab in more than 50% of the patients [93]. It should be noted that sustainable tumor control requires long term treatment with bevacizumab [94]. Because side effects of bevacizumab include hypertension, disrupted blood coagulation, embolism and kidney complications great caution should be exercised before starting therapy.

Another way to inhibit angiogenesis is by blocking the platelet-derived growth factor (PDGF) pathway. Besides having a proangiogenic function PDGF serves as a mitogen for Schwann and schwannoma cells. Therefore PDGF inhibitors have a direct effect on the schwannoma cells themselves as well. A drug capable of inactivating this pathway is imatinib mesylate (Glivec) [37,95]. The possibility that PDGF serves as a target for vestibular schwannoma treatment was first suggested by Altuna et al. [37,96]. They demonstrated that vestibular schwannomas express the PDGF Receptor-β and showed the ability of imatinib to alter cell cycle distribution and induce apoptosis in the vestibular schwannoma cell line HEI193. They additionally demonstrated that imatinib inhibited cell proliferation in HEI193 and in primary vestibular schwannomas cells. Yener et al. [97] confirmed the growth inhibitory effect of imatinib. They conducted angiogenesis assays on vestibular schwannomas. Imatinib proved to be effective in reducing the angiogenic activity. Ammoun et al. [98] compared imatinib with the

more potent PDGFR receptor inhibitor nilotinib (Tasigna). They found nilotinib to effectively inhibit proliferation of vestibular schwannoma cells at concentrations 6-10 times lower than imatinib. Additionally they demonstrated that a combination of nilotinib with selumetinib (AZD6244), a MAPK kinase inhibitor, even further inhibited cell proliferation.
Apart from antiangiogenic therapies other therapeutic agents have been tested. Lapatinib is a dual EGFR/ErbB2 inhibitor. In vitro studies have demonstrated that this drug achieves inhibition of cell proliferation in vestibular schwannoma cell cultures and it induces apoptosis in the HEI193 cell line [100,101]. This effect was endorsed by a phase II trial testing volume and hearing responses in NF2 patients. Results showed ≥15% tumor volume decrease in 4 out of 17 patients. Hearing was monitored in 13 patients, 4 of which experienced an improvement in pure tone average of at least 10 dB [107].
The recently developed histone deacetylase inhibitor AR42 capable of blocking the PI3K/AKT pathway proved to be a potent growth inhibitor of schwannoma- and meningeoma cells [103]. Targeting PDK1, which is a crucial activator of this pathway, can also inhibit AKT signalling in schwannoma cells [108] Yet another tyrosine kinase inhibitor tested for vestibular schwannoma therapy is erlotinib. It acts through HER-1/EGFR inhibition. Erlotinib showed to inhibit growth of vestibular schwannoma xenografts in nude mice [109]. Subsequent tests on the efficacy of erlotinib in 11 vestibular schwannoma patients [99] showed no radiographic or hearing responses but some patients did experience prolonged stable disease.
FRAX597 is an inhibitor of PAK, the kinase responsible for the phosphorylation of merlin (Figure 3). Licciulli et al. [104] found it to reduce proliferation in schwannoma cell lines and impair tumor development in an NF2 mouse model. Giovannini et al. showed that targeting the mTORC1 pathway with rapamycin (Sirolimus) leads to antagonization of tumorgenesis. This observation was made in in vitro as well as in vivo schwannoma models. They even seemed to induce tumor growth arrest in an NF2 patient [105] It should be noted that these observations are opposed by results of a trial describing no tumor resonse in 9 patients treated with mTOR inhibitor everolimus [102]
Finally there is the remarkable observation that plain aspirin is also associated with halted growth of vestibular schwannomas[106]. It is suggested that the COX2 inhibiting effect of aspirin dampens the pathologic immune response and its tumour promoting stimuli resulting in halted tumor progression. Table 3 provides an overview of target therapy tested for vestibular schwannomas.

Future prospects

To date a wide range of potential therapeutic targets for vestibular schwannoma treatment has been studied. Most drugs seem to induce an antiangiogenic or cytostatic response. An actual cytotoxic effect resulting in apoptosis has also been observed, for example with drugs

Table 3. Summary of research on targeted therapy

Drug	Target	Author (ref. nr.)	Main results
Bevacizumab	VEGF	Plotkin et al. [7]	Tumor shrinkage and mild hearing improvement in 9 out of 10 patients.
		Plotkin et al.[93]	Tumor shrinkage in >50% of 31 analysed patients
		Mautner et al. [5]	>40% tumor shrinkage in 2 out of 2 patients.
		Wong et al. [6]	Mouse model showing decrease of tumor vasculature after bevacizumab. Tumor growth decreased and the survival of treated mice extended by 50%.
Erlotinib	EGFR	Plotkin et al.[99]	No radiographic or hearing response in 11 patients.
Imatinib	PDGF	Altuna et al. [96]	In vitro study demonstrating apoptosis and inhibition of cell proliferation
		Yener et al. [97]	In vitro study demonstrating reduction of angiogenesis in tissue specimens of NF-2 related as well as sporadic tumors.
Lapatinib	EGFR/ErbB2	Ahmad et al. [100]	In vitro study demonstrating decrease of cell growth and proliferation in vestibular schwannoma cell cultures.
		Ammoun et al. [101]	In vitro study demonstrating decreased proliferation in a human schwannoma model
		Karajannis et al. [102]	Phase II trial demonstrating hearing an volume responses in lapatinib treated NF2 patients
AR42	PI3K/AKT	Bush et.al.[103]	Growth inhibition of schwannoma cells
FRAX597	PAK	Licciulli et al.[104]	In vitro study displaying reduced schwannoma cell proliferation and an in vivo experiment indicating impairment of tumor development.
Rapamycin	mTORC1	Giovannini[105]	Tumor growth arrest in one NF2 patient
Aspirin	COX-2	Kandatil et. al.[106]	Inverse association between aspirin use and tumor growth

targeting EGFR and PDGF. By simultaneously targeting EGFR and ErbB2 actual tumour shrinkage could be accomplished in a number of NF2 patients. This outcome emphasizes the potential effect of combining different therapies. The ideal would be a therapeutic regimen of drugs tailored to the gene- or protein expression pattern within each individual tumour. Such an approach would require analysis of tumour tissue, which is virtually impossible in non-surgically treated patients, but could be applied in a subgroup of patients suffering from tumour recurrence after surgery. The knowledge of having a good treatment alternative after surgery may also lessen the need for radical tumour extirpation, allowing more limited surgery with less morbidity.

Combining drugs with radiotherapy, a treatment approach already applied for different types of cancer, is another strategy worth considering. A recent study combing a c-Jun N-terminal kinase (JNK) inhibitor with gamma radiation[110] shows that the combination of these therapeutic strategies can be successful in vestibular schwannomas as well. Altogether these emerging therapeutic targets will help to further reduce the need for surgical intervention.

Conclusion

During the past years there has been a tremendous increase in knowledge of vestibular schwannoma biology. The mechanisms through which merlin carries out its functions are gradually elucidated. This process goes hand in hand with the identification of novel therapeutic targets. Up till now targeting angiogenesis seems the most successful pharmacological strategy but additional therapeutic options are emerging. Other tumor biological issues that deserve to be part of future research are the processes responsible for the variable growth pattern of these tumors, the discrepancies regarding the occurrence of (epi) genetic changes to *NF2,* and the potential involvement of additional genes and signalling pathways.

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