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REVIEW

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Current molecular understanding of central nervous system schwannomas

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Abstract

Background Schwannomas are tumors that originate from myelinating Schwann cells and can occur in cranial, spinal, and peripheral nerves. Although our understanding of the molecular biology underlying schwannomas remains incomplete, numerous studies have identified various molecular findings and biomarkers associated with schwannomas of the central nervous system (CNS). The development of these tumors is primarily linked to mutations in the *NF2* gene. Merlin, the protein encoded by *NF2*, is integral to several signaling pathways, including Ras/Raf/MEK/ERK, PI3K/Akt/mTORC1, Wnt/β-catenin, and the Hippo pathway.

Main body Recent research has also uncovered novel genetic alterations, such as the *SH3PXD2A::HTRA1* fusion gene, *VGLL*-fusions in intraparenchymal CNS schwannomas, and the *SOX10* mutation particularly in non-vestibular cranial nerve schwannomas. In addition to genetic alterations, research is also being conducted on gene expression and epigenetic regulation, with a focus on *NF2* methylation and post-transcriptional silencing by micro RNA. Furthermore, the advent of advanced techniques like single-cell sequencing and multi-omics analysis has facilitated rapid discoveries related to the tumor microenvironment and tumor heterogeneity in schwannomas.

Conclusion A deeper exploration of these molecular findings could clarify the mechanisms of schwannoma tumorigenesis and progression, ultimately guiding the development of new therapeutic targets. This review offers a comprehensive overview of the current molecular understanding of CNS schwannomas, emphasizing the insights gained from previous research, while addressing existing controversies and outlining future research and treatment perspectives.

Keywords Schwannoma, Central nervous system, Molecular, Genetic alteration, Gene expression, Epigenetics, Tumor microenvironment

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Introduction

Schwannomas are benign neoplasms that originate from the myelinating Schwann cells of the nerve sheath and can be pathologically classified as cellular Antoni A and hypocellular Antoni B areas. They can arise from cranial, spinal, and peripheral nerves. The majority of the schwannomas of the central nervous system (CNS) are vestibular schwannoma (VS), which arise from the vestibular division of the eighth cranial nerve [1]. These tumors account for approximately 8% of all intracranial tumors and nearly 80% of all cerebellopontine angle (CPA) masses [2, 3]. The majority of VS (95%) are sporadic and occur unilaterally; however, a genetic syndrome known as *NF2*-related schwannomatosis results in bilateral VS [4, 5]. Sporadic VS is most prevalent in individuals between the ages of 40 and 60 years, while NF2-associated VS, occurring as a part of *NF2*-related schwannomatosis, is more commonly diagnosed in younger individuals [6–8]. The majority of intracranial schwannomas are VS, while limited studies have focused on non-vestibular cranial nerve schwannoma such as those affecting the trigeminal, facial, and vagus nerves, resulting in a limited understanding of their molecular background. Furthermore, spinal schwannomas, which are considered extracranial CNS schwannoma, represent the most common primary spinal tumors, accounting for approximately one-third of all spinal tumors [9]. To date, several studies have identified genetic findings and molecular biomarkers of schwannomas of the CNS. However, the molecular biology underlying schwannomas is yet to be fully elucidated. In recent years, with the rapid improvement of genetic analysis techniques, including next-generation sequencing (NGS) and single-cell analysis, alongside technological advancements in bioinformatics to efficiently process large amounts of data, have enabled the gradual revelation of the molecular findings of schwannomas. This review aims to summarize the current comprehensive molecular biological understanding of CNS schwannomas, with particular focus on VS but also addressing other intracranial and spinal schwannomas.

Mechanism of schwannoma tumorigenesis and tumor progression

Although the fundamental factors driving schwannoma tumorigenesis and tumor progression in schwannomas are yet to be fully elucidated, several studies have started to uncover some of these mechanisms. The initial tumorigenic transformation of Schwann cells is primarily triggered by loss-of-function mutations in merlin due to biallelic inactivation of *NF2* [10]. These mutations in merlin, the protein involved in intercellular adhesion, render Schwann cells more susceptible to physical stress and injury. Following various types of nerve damage, such as compression, physical trauma, and chronic noise exposure, affected Schwann cells often undergo dedifferentiation, becoming immature and tumorigenic due to a failure to re-differentiate. These tumorigenic Schwann cells adopt a transcriptional state similar to that of reparative Schwann cells in the nerve injury. This is supported by single-cell RNA sequencing (scRNA-seq) analysis, suggesting that various nerve injury factors and subsequent nerve repair processes may promote schwannoma development [11, 12].

Schwann cell differentiation is regulated not only by Schwann cell-intrinsic programs but also by instructive signals from adjacent axons and the surrounding inflammatory environment, which controls the growth behavior of schwannomas [10, 13–16]. Single-cell multi-omic analysis of sporadic VS suggests that the development and proliferation of schwannomas is related to their interactions with the tumor microenvironment (TME). According to the Injury-like VS model proposed by Barrett et al., the initiation of VS tumor growth occurs through oncogenic events, followed by critical stressors such as nerve injury, prompting VS-associated tumor Schwann cells to adopt states reminiscent of repair process and antigen presentation [11]. CSF1 signaling facilitates VS tumor progression by recruiting myeloid cells, such as monocytes and macrophages. In addition, Liu et al. employed scRNA-seq to demonstrate that schwannomas comprise two distinct cell group populations, distinguished by their activation of neural crest or nerve injury pathways, which delineate the tumor cell state and the construction of the TME, thereby implicating TME in schwannoma progression [12].

Signaling pathways regulated by merlin

Merlin is a protein comprising 595 amino acids encoded by the *NF2* [17]. Merlin is composed of a relatively conserved N-terminal FERM (four-point-one, ezrin, radixin, moesin) domain, followed by a flexible coiled-coil domain and a C-terminal hydrophilic tail where phosphorylation occurs [18]. Merlin functions as a tumor suppressor and regulates tumor growth in its open state, while phosphorylation converts it to a less active, more closed state [19]. Merlin is involved in multiple oncogenic signaling pathways (Fig. 1), including Ras/Raf/MEK/ERK and PI3K/Akt/mTORC1 signaling, which are downstream of receptor tyrosine kinases [20–22]. Furthermore, it also inhibits the activation of yes-associated protein (YAP) in the Hippo pathway, which controls cell proliferation and apoptosis [23, 24].

Merlin can organize the plasma membrane by linking membrane-associated proteins to the actin cytoskeleton and regulating signaling from the extracellular milieu [25, 26]. One activation mechanism of merlin is a loss of contact-mediated inhibition of cell proliferation, which regulates tissue growth [27, 28]. The interaction

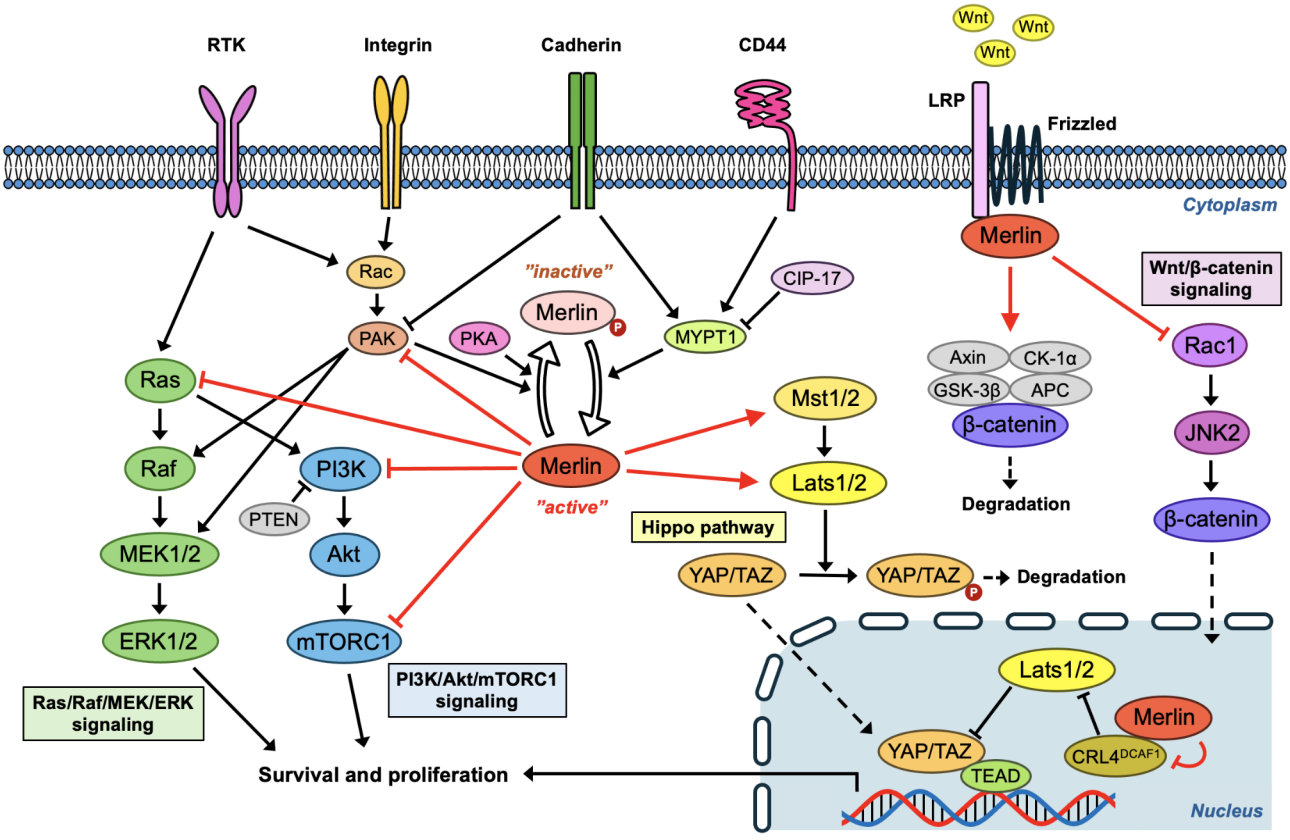

Fig. 1 Pathways regulated by merlin and related proteins. Merlin regulates multiple oncogenic signaling pathways, including Ras/Raf/MEK/ERK and PI3K/Akt/mTORC1 signaling, which are downstream of receptor tyrosine kinases, and Wnt/β-catenin signaling. Additionally, it inhibits the activation of Yes-associated protein (YAP) in the Hippo pathway, which is responsible for regulating cell proliferation and apoptosis. P21-activated kinase (PAK) and protein kinase A (PKA) inactivate merlin by phosphorylation. In contrast, merlin is dephosphorylated and activated by myosin phosphatase targeting subunit 1 (MYPT1)

of merlin with CD44, a transmembrane hyaluronic acid receptor, regulates cell growth arrest and proliferation in a contact inhibition-dependent manner [27, 29]. Proliferation signals from membrane-anchored integrins and receptor tyrosine kinases activate the signaling protein Rac, which in turn activates p21-activated kinase (PAK), then subsequently inactivates merlin by phosphorylation [30–33]. Additionally, merlin exerts a negative regulatory effect on the integrin-mediated Rac pathway at the membrane, thereby suppressing tumorigenesis [33, 34]. In contrast, cadherin-mediated adhesion inhibits PAK, thereby activating merlin by dephosphorylation [30–32]. Consequently, signals from both integrins and cadherins converge to induce conformational changes in merlin, facilitating cell cycle progression. In contrast, elevated levels of cyclic AMP, induced by the activation of G protein-coupled receptors (GPCRs), result in the phosphorylation of merlin by protein kinase A (PKA) [35]. Meanwhile, merlin is dephosphorylated and activated by myosin phosphatase targeting subunit 1 (MYPT1), and C-kinase potentiated protein phosphatase-1 inhibitor of 17 kDa (CPI-17) acts as an inhibitor of MYPT1 [33, 36–38]. Recent findings indicate that merlin deficiency impairs the cGAS-STING pathway, the DNA-sensing mechanism responsible for detecting tumor-derived DNA and inducing antitumor immunity [39]. Mutant merlin inhibits the cGAS-STING pathway by forming cellular condensates with IRF3 and TBK1, thereby impairing anti-tumor immune responses.

Ras/Raf/MEK/ERK signaling

The Ras/Raf/MEK/ERK signaling pathway, a type of mitogen-activated protein kinase (MAPK) signaling pathway, is involved in cell proliferation, differentiation, and the inhibition of apoptosis [40, 41]. Within this pathway, phosphorylation of Raf and MEK by PAK1 is required for effective Ras signaling [42, 43]. Given that merlin inhibits this Ras-mediated signaling pathway, merlin dysfunction allows for enhanced Ras signaling and an accelerated tumor growth in schwannomas [36]. In the study of human schwannoma cell lines and tumors, the overexpression of phosphorylated proteins in the Ras/Raf/MEK/ERK signaling was observed, indicating

molecular changes in the Ras/Raf/MEK/ERK components in schwannomas [21].

PI3K/Akt/mTORC1 signaling

The PI3K/Akt/mTORC1 signaling pathway similarly contributes to cell growth, differentiation, and apoptosis inhibition, and is upregulated in schwannomas [44]. The mammalian target of rapamycin complex 1 (mTORC1), a kinase complex that regulates cell growth, cell proliferation, cell motility, and cell survival, is a downstream signal of the PI3K/Akt pathway, with merlin acting as a negative regulator of mTORC1 [45, 46]. Furthermore, merlin suppresses PI3K/Akt signaling by directly binding and inhibiting the stimulatory activity of PIKE-L on PI3K [47]. Another negative regulator of this signaling pathway is phosphatase and tensin homolog (PTEN) [48]. Cayé-Thomasen et al. reported elevated PTEN expression in sporadic VS, suggesting that upregulation of PTEN compensates for the lack of merlin inhibition [49].

Wnt/β-catenin signaling

The Wnt/β-catenin signaling plays a vital role in development and disease progression and is required in most embryonic developmental processes in invertebrates and vertebrates [50–53]. However, abnormalities in this signaling cause various types of tumors [54, 55]. The activation of the Wnt/β-catenin signaling has also been observed in human schwannoma cells, demonstrated by the upregulated expression of Wnt target genes *c-myc* and *cyclin D1* [56]. When the Wnt/β-catenin signaling is activated, β-catenin binds to a destruction complex consisting of axin, casein kinase-1 alpha (CK1α), glycogen synthase kinase-3 beta (GSK3β), and adenomatous polyposis coli (APC), resulting in its subsequent degradation [57]. Merlin binds to the lipoprotein receptor-related protein, thereby inhibiting the phosphorylation of the receptor and leading to the accumulation of β-catenin within the cytoplasm [58]. In addition, merlin downregulates Rac1 and inactivates c-Jun N-terminal kinase 2 (JNK2), thereby preventing the transport of β-catenin to the cell nucleus [59]. In this way, merlin regulates cell proliferation; however, its dysfunction activates the Wnt/β-catenin signaling, which is associated with tumor development.

Hippo pathway

The Hippo pathway plays a role in regulating cell number, influencing processes such as cell proliferation, cell death, and cell differentiation [60]. Mutations affecting this pathway have been associated with various types of cancer [61]. Similarly, the Hippo pathway is inactivated by the downregulation of merlin in schwannomas [62]. A previous study showed that inactivation of the Hippo pathway in Schwann cells results in the development of multiple schwannomas in mice [63]. Merlin initiates the Hippo pathway and promotes the degradation of YAP and its homologous protein—transcription coactivator (TAZ) [24, 64]. In the inactivated state of merlin, YAP/TAZ translocate into the nucleus, where they bind to TEA domain family members (TEAD) and stimulate the transcription of growth-promoting and antiapoptotic genes, thus contributing to VS cell proliferation [65, 66]. In the nucleus, merlin also binds to DCAF1 and inhibits the E3 ubiquitin ligase CRL4. Furthermore, in the absence of merlin, the derepressed $CRL4^{DCAF1}$ complex promotes YAP activation by inhibiting the Hippo pathway kinases Lats1/2, thereby promoting cell proliferation [67–70].

Receptor tyrosine kinases

Receptor tyrosine kinases are crucial molecules that participate in intracellular signaling pathways, including those involved in cell proliferation. They play a significant role in the development and progression of sporadic VS and have the potential to be a therapeutic target [71]. Notable examples of receptor tyrosine kinases in sporadic VS include vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), ErbB family members, and hepatocyte growth factor (HGF). In schwannomas, the dysfunction of merlin results in the suppression of Semaphorin 3 F, which in turn enhances tumor angiogenesis via VEGF signaling [72]. VEGF expression is markedly elevated in sporadic VS, indicating that VEGF-induced tumor growth is at least partially mediated by angiogenesis promotion [73–76]. PDGF is a growth factor that is primarily involved in the regulation of mesenchymal cell migration and proliferation. Specifically, the overexpression of PDGFR is observed in schwannomas and is associated with tumor growth, making it a potential therapeutic target [20, 77–79]. The ErbB family proteins are highly expressed in sporadic VS, and their inhibition has been shown to yield therapeutic effects on tumor growth [80–82]. The hepatocyte growth factor receptor (c-MET) is a glycosylated receptor tyrosine kinases that activates oncogenic pathways such as RAS and PI3K [83]. Dilawi et al. suggested the possible crosstalk between the c-MET and VEGF-A in sporadic VS and found that both c-MET and VEGF-A are significantly overexpressed in these tumors [84].

Genetic alteration

NF2 mutation

The previous studies for genetic alterations in sporadic schwannomas are summarized in Table 1. The most prevalent mutations of the *NF2*, located on chromosome 22q12.2, account for 53–76% of sporadic VS cases [85–92]. However, these variations in mutation detection, especially in *NF2*, are to some extent dependent on

Table 1 Summary of previous studies on genetic alterations in sporadic schwannomas

Author, Year	Sample size	Analysis methods	Summary of results
Mohyuddin et al., 2002 [91]	VS ($n=28$)	Targeted exon sequencing and microsatellite marker analysis	*NF2* mutation was identified in 75% of VSs and 22 q LOH in 75% of VSs.
Bian et al., 2005 [101]	Schwannomas, ($n=54$ [vestibular, $n=36$; spinal, $n=18$])	Microsatellite marker analysis	43% of schwannomas showed 22q LOH
Aarhus et al., 2010 [87]	VS ($n=25$)	RT-PCR and DNA sequencing	76% of VSs had *NF2* mutations
Hadfield et al., 2010 [88]	VS ($n=104$)	DNA sequencing and a combination of microsatellite marker analysis with either CGH or MLPA	*NF2* mutation was identified in 66% of VSs, and 22 q LOH occurred in 56% of VSs
Lee et al., 2012 [90]	VS ($n=30$)	DNA sequencing and microsatellite marker analysis	*NF2* mutation was identified in 53% of VSs, and 22 q LOH occurred in 30% of VSs
Pećina-Slaus et al., 2012 [102]	Schwannomas ($n=16$ [vestibular, $n=15$; spinal, $n=1$])	Microsatellite marker analysis	The results regarding *NF2* showed 7 of the 16 heterozygous patients with allelic losses (43.75%)
Torres-Martin et al., 2013 [153]	VS ($n=31$)	PCR/dHPLC and MLPA, as well as a microsatellite marker analysis	*NF2* mutation was identified in 74% of VSs and 22 q LOH in 52% VSs
Oh et al., 2015 [86]	Schwannomas ($n=82$ [peripheral, $n=38$; spinal, $n=44$])	DNA sequencing	*NF2* mutation was identified in 55%, *LATS1* mutations in 2%, and *LATS2* mutations in 1% of schwannomas.
Agnihotri et al., 2016 [85]	Schwannomas ($n=126$ [vestibular, $n=64$; spinal, $n=62$])	Whole-exome sequencing and targeted exon sequencing	*NF2* mutation was identified in 57% of schwannomas. Other identified mutations included *ARID1B* (18%), *ARID1A* (14%), *DDR1* (11%), *TSC1* (9%), *CAST* (8%), *ALPK2* (8%), *LZTR1* (8%), *TSC2* (7%), and Table 3 (3%).
Håvik et al., 2018 [89]	VS ($n=46$)	Whole-exome sequencing and microsatellite instability testing	76% of VSs had *NF2* mutation, and none of the tumors showed microsatellite instability.
Williams et al., 2023 [107]	Schwannomas ($n=77$ [vestibular, $n=19$; non-vestibular cranial nerve, $n=8$; spinal, $n=33$; soft tissue, $n=16$; unknown, $n=1$])	DNA sequencing	*SOX10* indel mutation was identified in 28.5% of schwannomas.
Terry et al., 2023 [119]	Segmental schwannomatosis ($n=2$)	DNA sequencing	Somatic mosaic *SOX10* indel mutation was identified in 2 cases of segmental schwannomatosis.
Wali et al., 2023 [118]	Orbital schwannoma ($n=1$)	DNA sequencing	*SOX10* mutation was identified in orbital schwannoma with plexiform growth.

VS: vestibular schwannomas; CGH: conventional comparative genomic hybridization, MLPA: multiplex ligation-dependent probe amplification, PCR: polymerase chain reaction, dHPLC: denaturing high-performance liquid chromatography

the genetic analysis method used. *NF2* is a tumor suppressor gene, comprising 16 constitutive exons and one alternatively spliced exon. Its discovery was first reported in 1993 [93, 94]. The most prevalent types of *NF2* germline mutations in *NF2*-associated schwannomas are truncating mutations, as a results of frameshift and nonsense mutations [88]. Similarly, truncating mutations of *NF2* are also most prevalent in sporadic schwannomas [88, 90, 95]. Previous studies have demonstrated that somatic mutations affecting both alleles of *NF2* result in the inactivation of this tumor suppressor gene, triggering tumorigenesis in sporadic VS [96, 97]. This phenomenon is encapsulated in the "two-hit" theory, also known as the Knudson hypothesis: the tumorigenesis of sporadic schwannomas is attributed not only to somatic mutations in *NF2* but also to a second hit—loss of heterozygosity (LOH) due to partial or complete loss of 22q—which further contributes to *NF2* inactivation and tumorigenesis [98, 99]. Carlson et al. employed whole-exome sequencing, mate-pair analysis, and RNA sequencing to profile genome-wide alterations in sporadic VS, revealing that somatic mutations were present in both alleles of all tumors tested [100]. Furthermore, the authors demonstrated that the mechanisms of damage to the second allele (second hit) were diverse: a loss of chromosome 22 occurred in 56% of tumors, while another mutation in *NF2* was observed in 22%, and copy-neutral 22q LOH in 17% of tumors. Consequently, the "two-hit" theory of *NF2* can account for a significant proportion of sporadic schwannomas tumorigenesis. However, it should be noted that 22q LOH in sporadic VS is not universally observed, accounting for only 30–75% of cases [85, 88–92, 101, 102].

Genetic mutations other than *NF2*

In addition to *NF2* mutations, other genetic mutations including *ARID1A*, *ARID1B*, *DDR1*, *LATS1*, and *LATS2* have also been identified in both VSs and spinal schwannomas [85, 86]. *LATS1/2* are genes that code for serine threonine kinase genes positioned downstream of *NF2* and act as a negative regulator of the yes-associated protein (YAP) oncogene in the Hippo signaling pathway [86, 103]. Furthermore, in a mouse model, *Lats1/2* were reported to act as a tumor suppressor gene, and the

deletion of these gene resulted in the schwannomagenesis [63]. *ARID1A/B* are histone-modifying genes [104], and *DDR1* (discoidin domain receptor tyrosine kinase 1) is a receptor tyrosine kinase activated in lung cancer and other tumors [105, 106]. These genetic mutations are less frequently observed with few recurrences in sporadic schwannomas than *NF2*, and their specific role in schwannoma biology and association with clinical presentation remains unknown.

Indel mutation of *SOX10* gene

A recent study identified *SOX10* in-frame insertion/deletion (indel) mutations as genetic drivers of nearly one-third of schwannomas [107]. These *SOX10* indel mutations were highly enriched in schwannomas arising from non-vestibular cranial nerves, including the trigeminal, facial, and vagus nerves. In contrast, they were absent in VSs driven by *NF2* mutation. The *SOX10* gene encodes a homeobox transcription factor that is critical for differentiation and maturation of Schwann cells into a myelinating cell state [108–112]; the transcription factor SOX10 belongs to a family of 20 SRY (sex-determining region Y)-related high mobility group box-containing (SOX) proteins [113].

Bremer et al. provided the first evidence that the transcription factor SOX10 is essential for Schwann cell function in the peripheral nervous system, highlighting its necessity for differentiation and maintenance of the differentiated state [114]. Mice with *SOX10* mutation do not form Schwann cells or die from peripheral neuropathy [108, 109], and loss of SOX10 function is also key to the pathology of human Merlin-null schwannomas [14]. In addition, SOX10 is also known to cause Waardenburg syndrome type 4 (WS4) which is characterized by the association of Waardenburg syndrome (variable spectrum of sensorineural hearing loss and pigmentary abnormalities of the hair and skin) and Hirschsprung disease (aganglionic megacolon) [108, 115–117]. WS4 is an autosomal recessive neurocristopathy, predominantly resulting from truncating loss-of-function germline mutation scattered throughout the *SOX10* gene, though whole gene deletions can occur on occasion. However, the *SOX10* indel mutations observed in these schwannomas are somatic mutations and do not represent loss-of-function, indicating a different functional mechanism for *SOX10* in their pathogenesis [107].

The occurrence of schwannomas with somatic *SOX10* mutations is exceedingly scarce. Williams et al. documented 22 cases in their cohort, with only two additional cases linked to segmental schwannomatosis and one case of orbital schwannoma [107, 118, 119]. Wali et al. postulated a potential association between plexiform schwannoma—one specific subtype of schwannoma—and *SOX10* mutations [118]. Although a tendency for postoperative recurrence has been indicated in *SOX10*-mutant schwannomas [107], further investigation into the clinical features and mechanism of pathogenesis associated with *SOX10* mutations is necessary, particularly in non-vestibular cranial nerve schwannomas.

Somatic mosaicism and germline mutations in sporadic schwannomas

The genetic alterations described so far are primarily somatic mutations detected in schwannoma tumor samples, indicating that sporadic schwannomas have a low rate of association with germline pathogenic variants. However, patients with apparently sporadic schwannomas may in fact harbor undiagnosed *NF2*-related schwannomatosis or NF2 mosaicism [95, 120]. Although such cases are rare, particularly outside the young population, genetic analyses using lymphocyte DNA from patients under the age of 25 years have revealed an identifiable genetic predisposition in 29% of these patients [121].

In a genome-wide association study (GWAS) involving 911 lymphocyte DNA samples of sporadic VS cases and 5,500 control lymphocyte DNA samples, Sadler et al. identified an association between the 9p21.3 region and the risk of sporadic VS. They proposed that LOH at the 9p21.3 locus may represent a mutational hit in schwannomas in which *NF2* mutation is not observed [122].

Besides being a principal feature of *NF2*-related schwannomatosis, schwannomas are also principal in another hereditary tumor disease—Schwannomatosis [123]. Schwannomatosis was recognized as a distinct disorder only recently, and is still very difficult to distinguish schwannomatosis from *NF2*-related schwannomatosis or sporadic schwannomas. The main distinction is that it consists of multiple schwannomas without associated vestibular schwannoma typical for *NF2*-related schwannomatosis. Most schwannomas in schwannomatosis are also caused by a *NF2* mutation, nevertheless a novel gene candidate *INI1/SMARCB1*, is indicated to be responsible for a part 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.

Gene expression

A number of gene expression analyses have been conducted in schwannomas; however, few have been consistently reproducible. These studies have identified both increased and decreased expression of various genes in the context of tumor development, tumor growth, and hearing loss [41, 44, 49, 87, 92, 124–127]. A summary of previous studies for gene expression of sporadic schwannomas can be found in Table 2. The tumor suppressor gene *CAV1* has been reported to be downregulated and

Table 2 Summary of previous studies on gene expression in sporadic schwannomas

Author, Year	Sample size	Analysis methods	Summary of results
Welling et al., 2002 [44]	VS ($n=7$) and normal vestibular nerve ($n=1$)	Microarray analysis and RT-PCR	*LUCA-15* was downregulated in VSs
Stankovic et al., 2009 [125]	VS ($n=13$)	Microarray analysis	*PEX5L* was downregulated in VSs with poor hearing
Aarhus et al., 2010 [87]	VS ($n=25$) and normal tibial nerves ($n=3$)	Microarray analysis	*CAV1* was downregulated in VSs
Torres-Martin et al., 2013 [92]	VS ($n=31$) and control nerves ($n=9$)	Microarray analysis and RT-PCR	*MET* and associated genes, such as *ITGA4/B6*, *PLEXNB3/SEMA5*, and *CAV1*, were downregulated in VSs.
Agnihotri et al., 2014 [41]	Schwannomas ($n=49$) and normal vestibular ($n=7$) nerves	Microarray analysis	PI3K/AKT/mTOR signaling network was upregulated.
Agnihotri et al., 2016 [85]	Schwannomas ($n=126$ [vestibular, $n=64$; spinal, $n=62$])	Bulk RNA sequencing and RT-PCR	*SH3PXD2A::HTRA1* fusion gene was identified in 10% of schwannomas.
Sass et al., 2017 [126]	VS ($n=16$)	Microarray analysis	109 genes were downregulated in relation to tumor growth rate. Generated functional networks underlined the importance of the PI3K family.
Taule-Sivertsen et al., 2021 [131]	VS ($n=121$)	RT-PCR	*SH3PXD2A::HTRA1* fusion gene was identified in 0.8% of Norwegian patients with VS.
Ogasawara et al., 2023 [133]	MPNST of the trigeminal nerve ($n=1$)	RT-PCR	*SH3PXD2A::HTRA1* fusion gene was identified in MPNST of the trigeminal nerve caused by the malignant transformation.
Breun et al., 2023 [129]	Normal vestibular nerves ($n=1$), VS ($n=49$), recurrences of VS ($n=8$), NF2-associated VS ($n=5$), and recurrences of NF2-associated VS ($n=6$)	RT-PCR	*MACC1* expression was significantly higher in sporadic VS compared to NF2-associated VS, and *MACC1* expression was correlated with deafness.
Fu et al., 2023 [128]	VS ($n=77$) and normal nerves ($n=20$)	Microarray analysis	*CCND1*, *CAV1*, *GLI1*, *SOX9*, *LY86*, *TLR3*, *TREM2*, and *C3AR1* were identified as DEG.
Lee et al., 2024 [132]	Schwannomas ($n=215$ [intracranial, $n=75$; somatic soft tissue, $n=62$; spinal, $n=38$; body cavity or head and neck, $n=20$; and visceral, $n=13$])	RT-PCR	*SH3PXD2A::HTRA1* fusion gene was identified in 13% of schwannomas.
Schmid et al., 2024 [130]	Intraparenchymal CNS schwannomas ($n=20$ [supratentorial, $n=15$; infratentorial, $n=4$; and spinal, $n=1$])	Bulk RNA sequencing by NGS	Fusion genes of *CHD7::VGLL3* (52.9%) and *EWSR1::VGLL1* (29.4%) was identified in intraparenchymal CNS schwannomas.

VS: vestibular schwannoma; RT-PCR: reverse transcription polymerase chain reaction, MPNST: Malignant peripheral nerve sheath tumor, DEG: differentially expression gene, CNS: central nervous system, NGS: next-generation sequencing

classified as one of the differentially expression genes (DEG) in sporadic VS [87, 92, 128]. Breun et al. demonstrated that *MACC1*, a gene implicated in angiogenesis, cell proliferation, invasiveness, cell motility, and metastasis in solid malignant cancers, was significantly expressed in sporadic VS and had a noteworthy correlation with hearing loss [129]. A pathway analysis of the genes with altered expression suggest that genes related to apoptosis, growth, and cell proliferation are downregulated, highlighting the pivotal role of the PI3K family of genes in this process [41, 126]. In particular, *SH3PXD2A::HTRA1* fusion gene has recently been identified as a pathogenic genetic alteration for schwannomas and is a potential therapeutic target. Notably, the latest study has identified *VGLL*-fusions in intraparenchymal CNS schwannomas [130].

SH3PXD2A::HTRA1 fusion gene

In a comprehensive molecular analysis conducted by Agnihotri et al., a novel *SH3PXD2A::HTRA1* fusion gene was identified in 10% of schwannomas, with a male predilection for fusion gene-positive VS [85]. This fusion resulted from a balanced 19-Mb chromosomal inversion on chromosome 10q. The fusion gene product was shown to promote tumorigenesis in vitro and in vivo through increased phosphorylation of ERK [85]. Furthermore, it is involved in the activity of the MAPK signaling pathway, suggesting the possibility of molecular targeted therapy using MEK inhibitors [40]. The prevalence of schwannomas harboring *SH3PXD2A::HTRA1* fusion gene has been reported as 10–13% among North American patients [85], whereas lower incidences of 0.8% among Norwegians and 1.3% among Taiwanese patients have been documented [131, 132]. Ogasawara et al. reported the detection of a *SH3PXD2A::HTRA1* fusion gene in a recurrent trigeminal schwannoma that had undergone malignant transformation into a malignant peripheral nerve sheath tumor (MPNST) [133]. Lee et al. observed a higher prevalence of schwannomas of peripheral nerve origin with a fusion gene, which exhibited a "serpentine" palisading pattern in histopathological examinations [132]. However, the clinical characteristics, including

ethnic group and site of origin, as well as their correlations with prognosis, remain inadequately understood.

VGLL-fusions in intraparenchymal CNS schwannomas

Schmid et al. analyzed 20 cases of intraparenchymal CNS schwannomas to identify fusion genes of *CHD7::VGLL3* (52.9%) and *EWSR1::VGLL1* (29.4%) [130]. They revealed that these rare tumors exhibit a distinct DNA methylation profile, frequently harbor *VGLL1* or *VGLL3* fusions, and demonstrate favorable clinical behavior with no recurrence during follow-up. While *VGLL3* and *VGLL1* are associated with the Hippo pathway, the activation of this pathway via *VGLL*-fusions and its involvement in the pathogenesis of intraparenchymal CNS schwannomas remains unclear.

Epigenetic regulation

The term "epigenetics" is used to describe the mechanisms that regulate gene activity without directly affecting the DNA sequence. These mechanisms include DNA methylation and microRNA (miRNA). DNA methylation represents a stable form of gene silencing at the transcriptional level, whereas miRNAs inhibit gene expression at the post-transcriptional level. The epigenetic mechanisms of gene expression regulation have been established for various tumors and are also being elucidated in sporadic VS [134]. A comprehensive epigenetic analysis has recently revealed that VSs are comprised of two molecular groups: neural crest and immune-enriched schwannoma, with radiotherapy inducing immune-enriched schwannoma through metabolic and epigenetic reprogramming [12].

DNA methylation

A subset of sporadic VS cases exhibit wild-type *NF2* (24–47%), suggesting that not all sporadic VS cases have confirmed mutations in the *NF2* [85–92]. It has been suggested that the underlying cause of the merlin dysfunction observed in sporadic VS cases lacking discernible genetic alterations may be attributed to the inhibition of gene transcription resulting from aberrant methylation of the CpG island in the promoter region of the *NF2*. However, studies on the methylation of *NF2* have yielded inconsistent results, and the role of DNA methylation in the pathogenesis of sporadic VS remains unclear. Kino et al. demonstrated the presence of a CpG site in the region of *NF2* that is involved in transcriptional repression of the gene and identified abnormal methylation of *NF2* in 14 of 23 (61%) cases of sporadic VS [135]. Furthermore, the methylation of *NF2* resulted in a reduction in mRNA expression. Gonzalez-Gomez et al. revealed that hypermethylation of *NF2* was observed in 6 of 31 (19%) cases of sporadic VS, yet it was not correlated with mutation or deletion of *NF2* [136]. Conversely, Kullar et al. conducted the most comprehensive study to date, employing the more sensitive pyrosequencing method. The findings indicated that only four of the 39 sporadic VS samples exhibited low levels of methylation of *NF2*, with no instances of high levels of methylation [137]. Moreover, Koutsimpelas et al. observed no methylation of *NF2* in any of the 23 tumors examined, leading them to conclude that inactivation of *NF2* by hypermethylation of CpG islands is a rare occurrence [138]. Further studies are necessary to confirm this conclusion, however, the current evidence suggests that the methylation of *NF2* appears to play little or no role in the pathogenesis of VS [90, 139].

Consequently, investigations have also been conducted into the methylation of other genes. Gonzalez-Gomez et al. proposed that the methylation of *THBS1*, *TP73*, *MGMT*, and *TIMP-3* may be involved in the formation of sporadic VS [136]. Lassaletta et al. demonstrated a significant correlation between CASP8 and RASSF1A gene methylation, with hypermethylation of *RASSF1A* being correlated with tumor growth [140]. The RASSF1A gene has been identified as a high-risk tumor suppressor gene that is epigenetically inactivated in many cancers and acts through the Ras-dependent apoptotic pathway [141]. Torres-Martin et al. examined genome-wide DNA methylation patterns in sporadic VS and observed a trend toward hypomethylation [142]. Moreover, they demonstrated hypomethylation of CpG islands of homeobox (HOX) genes situated in four clusters of the genome.

Methylation subtypes illuminate cell origin, tumor heterogeneity, and tumor phenotypes. To date, three comprehensive genome-wide DNA methylation analyses have been conducted on sporadic schwannomas [12, 85, 107]. Agnihotri et al. conducted a DNA methylation clustering analysis of sporadic schwannomas, which resulted in the classification of these tumors into two groups: VSs and spinal schwannomas [85]. This was the first study to identify distinct molecular subgroups of schwannomas associated with anatomical location. Williams et al. identified the *SOX10* indel mutation as a novel driver gene for sporadic schwannomas [107]. They demonstrated that the schwannomas with the *SOX10* indel mutation exhibited a distinct DNA methylation profile compared to those with the *NF2* mutation, indicating that schwannomas may manifest distinct subtypes contingent on the underlying driver genes. Liu et al. distinguished between two categories of sporadic schwannomas: neural crest schwannomas and immune-enriched schwannomas [12]. They demonstrated that the DNA methylation profiles differed between the two groups. Among the 2,000 differentially methylated probes, the hypomethylated probe ontologies in immune-enriched schwannomas were found to be related to the blood and immune systems.

Table 3 Summary of previous studies on miRNA expression in sporadic schwannomas

Author, Year	Sample size	Analysis methods	Summary of results
Cioffi et al., 2010 [151]	VS ($n=8$), normal vestibular nerves ($n=9$), and greater auricular nerves ($n=5$)	RT-PCR	Upregulation of miR-21 was observed, which correlated with a decreased level of PTEN.
Saydam et al., 2011 [145]	VS ($n=10$) and normal peripheral nerves ($n=2$)	Microarray analysis and RT-PCR	12 miRNAs including miR-7 were identified as the most altered miRNAs.
Torres-Martin et al., 2013 [153]	VS ($n=16$) and normal nerves ($n=3$)	Microarray analysis and RT-PCR	Downregulation of 174 miRNAs were identified including miR-10b, miR-206, miR-183 and miR-204, as well as the upregulation of miR-431, miR-221, miR-21 and miR-720.
Torres-Martin et al., 2015 [142]	VS ($n=36$), non-vestibular cranial nerve schwannomas ($n=4$), and normal nerves ($n=5$)	Microarray analysis	MiRNA-21 was upregulated in schwannomas.
Li et al., 2016 [148]	VS ($n=95$) and normal vestibular nerves ($n=79$)	RT-PCR	Downregulation of miR-1 is associated with increased tumor volume in VSs.
Yan et al., 2019 [160]	VS ($n=29$ [cystic VS, $n=17$; solid VS, $n=12$)	Microarray analysis and miRNA sequencing	Genes of the FOXO family were identified as miRNAs responsible for the cystic growth of schwannomas.
Lei et al., 2019 [146]	VS ($n=26$) and normal nerves ($n=5$)	Microarray analysis	MiR-181a-5p, miR-106-5p, and miR-34a-5p were the top three DEmiRNAs that covered most DEmRNAs.
Xiao et al., 2022 [152]	VS ($n=56$) and normal vestibular nerves ($n=42$)	RT-PCR	BRCAT54 acts as a sponge for miR-21, thereby inhibiting cell proliferation in VSs.
Fujita et al., 2023 [157]	VS ($n=13$ [good hearing group, $n=6$; poor hearing group, $n=7$])	Bulk RNA sequencing by NGS	MiR-431-5p was upregulated in the poor hearing group of VSs.
Litwiniuk-Kosmala et al., 2024 [159]	VS ($n=20$ [small tumors, $n=10$; and large tumors, $n=10$])	Bulk RNA sequencing by NGS	MiR-7, miR-142 (-3p and −5p), miR-155, miR-342, miR-1269, miR-4664, and miR-6503 were upregulated in large VSs.
Litwiniuk-Kosmala et al., 2024 [158]	VS ($n=19$ [good hearing group, $n=8$; and poor hearing group, $n=11$])	Bulk RNA sequencing by NGS	21 miRNAs upregulated in VS with preoperative hearing loss were identified.

VS: vestibular schwannoma; DEmiRNA: differentially expressed miRNA: DEmRNA: differentially expressed mRNA: NGS: next-generation sequencing

microRNA (miRNA)

MiRNA is a small non-coding RNA approximately 21 to 23 nucleotides in length that regulates gene expression at the post-transcriptional level by binding to and silencing complementary mRNAs [143]. MiRNAs are critical in tumorigenesis, as they can act as either tumor suppressor genes or oncogenes, depending on whether they are upregulated or downregulated. Studies of miRNA expression in sporadic VS have identified several differentially expressed miRNAs associated with prognostic and clinical factors [144–146]. Moreover, miRNAs have been identified as valuable diagnostic and prognostic tools, as well as potential candidates for therapeutic targets [145, 147–149]. The previous studies of miRNA expression in sporadic schwannomas are summarized in Table 3.

MiR-21 is one of the most commonly studied miRNAs associated with tumor growth due to its increased expression. It targets the antioncogene *PTEN*, and increased levels of miR-21 have been found in both VS and human cholesteatoma [142, 150]. Cioffi et al. employed reverse transcriptase-polymerase chain reaction (RT-PCR) to analyze miR-21 upregulation in eight sporadic VS cases compared to nine normal vestibular nerves and five normal greater auricular nerves [151]. They observed a consistent upregulation of miR-21, which correlated with a decreased level of PTEN. Xiao et al. demonstrated that BRCAT54, a long noncoding RNA (lncRNA) in lung cancer, acts as a sponge for miR-21, thereby inhibiting cell proliferation in sporadic VS [152].

Torres-Martin et al. analyzed the miRNA expression profile of 16 VS samples using microarray technology, identifying deregulation of 174 miRNAs, including miR-10b, miR-206, miR-183 and miR-204, as well as the upregulation of miR-431, miR-221, miR-21 and miR-720, among others [153]. Additionally, they discovered that the majority of the miRNAs that were upregulated were located in the chromosomal region of 14q32. In a separate microarray study, Saydam et al. identified 12 miRNAs, with miR-7 being the most significantly altered miRNAs in sporadic VS [145]. MiR-7 has been demonstrated to be downregulated in several other tumors [154, 155], while, both cell line experiments and animal tumor models demonstrated that its upregulation suppresses tumor growth.

The recent advent NGS for RNA has enabled high-throughput and detailed analysis of the entire transcriptome, including miRNA expression profiles in tissue samples [156]. Fujita et al. employed NGS to RNA extracted from VS cells and identified miR-431-5p as a differentially expressed miRNA associated with preoperative hearing loss in the poor hearing group [157]. Litwiniuk-Kosmala et al. employed NGS to identify 21 miRNAs upregulated in VS with preoperative hearing loss; these belonged to the miR 449a/b, miR 15/16 – 1, and hypoxamiR families [158]. They also analyzed miRNAs

associated with VS size and identified eight upregulated miRNAs (miR-7, miR-142 -3p, miR-142-5p, miR-155, miR-342, miR-1269, miR-4664, and miR-6503) and miR-204, which were downregulated in large VS (stage II–IV Koos classification) compared to small VS (stage I Koos classification) [159]. Yan et al. compared the microRNA expression profiles of solid and cystic VS and identified 55 microRNAs responsible for the cystic formation of the tumor. They found that these microRNAs regulate *PTEN*, *FOXO1*, *FOXO3*, *VEGFA*, and *SIRT1* [160].

As previously stated, Litwiniuk-Kosmala et al. demonstrated that miR-204 expression was downregulated in larger VS [159]. MiR-204 is a well-known tumor suppressor, with reduced expression noted in numerous types of cancers, including MPNST [161]. In addition, Li et al. demonstrated that, in sporadic VS, decreased miR-1 expression is associated with increased tumor volume, indicating that miR-1 inhibits tumor growth by targeting VEGFA [148].

Tumor microenvironment (TME)

The TME is a complex ecosystem comprising various cell types, including immune cells, vascular system cells, fibroblasts, and cancer stem cells, in addition to tumor cells. These cells interact and influence tumor behavior, playing a role in the development and progression of schwannomas [162]. Tumor-associated macrophages (TAMs) are involved in the regulation of proliferation and immune responses. Several studies have revealed an association between TAM infiltration and tumor progression in schwannomas [163, 164]. TAMs are categorized into pro-inflammatory M1 and anti-inflammatory M2 macrophages. In particular, the M2 macrophages appear to be linked to tumor progression and angiogenesis in schwannomas [165, 166]. In contrast to previous studies, Perry et al. reported the association between M1 macrophages and tumor progression after subtotal resection of VS [167]. Considering these findings, the function of TAMs in schwannomas remains controversial.

Recent studies using scRNA-seq have emerged as reliable methodologies for uncovering the association between schwannomas and TME. ScRNA-seq for sporadic VS was first reported in 2022 [168, 169]. Xu et al. and Yidian et al. performed scRNA-seq on three cases of VS each, revealing an interaction between schwannoma cells and TME [168, 169]. TAMs are reported to comprise 50–70% of all proliferating cells in situ [170], confirming that, at the single-cell level, VS is mainly composed of myeloid cells and schwannoma cells. Barrett et al. not only performed scRNA-seq on 15 VSs, but also performed single-cell assay of transposase accessible chromatin sequencing (scATAC-seq) on 6 VSs for the first time [11]. They identified a mechanism of TME associated with schwannoma development and progression, in which myeloid cells are induced by CSF1 signaling from injury-like Schwann cells to cause tumor growth. As mentioned previously, Liu et al. employed scRNA-seq and DNA methylation analysis to categorize schwannomas into two groups: neural crest and immune-enriched, characterized by macrophage and lymphocyte infiltration [12]. However, despite these novel findings, evidence regarding the TME in schwannomaws remains contentious, with findings from previous studies occasionally presenting contradictory results; thus, the tumor biology of the TME in schwannomas remains poorly understood [171].

Inflammation

Inflammatory responses contribute markedly to tumor growth and hearing loss in VS, with cyclooxygenase-2 (COX-2), cytokines/chemokines, and the nuclear factor kappa-B (NF-κB) playing pivotal roles in this process [170, 172]. Mutations in *NF2* activate the Hippo pathway, with the effector molecule YAP subsequently promoting COX-2 translation. Prostaglandin E2 (PGE2), which is metabolized by COX-2, exerts diverse effects on cell proliferation, apoptosis, angiogenesis, inflammation, and immune surveillance [173]. Hong et al. found that COX-2 was highly expressed in both sporadic VS and NF2-associated VS (15 cases each), particularly noting an association between COX-2 expression and higher tumor growth rates [174]. Kandathil et al., in their investigation of sporadic VS, found that 33 of 81 patients (41%) taking aspirin—a known COX-2 inhibitor—exhibited tumor growth, compared to 154 of 266 patients (58%) of nonaspirin users, suggesting that aspirin may have an inhibitory effect on the growth of sporadic VS [175]. In contrast, Marinelli et al. conducted a study of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) in users versus non-users in the context of sporadic VS. The results indicated that these drugs were not significantly correlated with tumor growth [176]. Moreover, the most recent meta-analysis has demonstrated that aspirin does not affect tumor growth in sporadic VS [177].

Chemokines represent a specific family of cytokines that induce the migration of immune cells toward one another. CXCR4, a chemokine receptor, is known to recruit blood stem cells and increase tumor cell growth and invasiveness, and has been reported to be involved in tumor growth in sporadic VS [178]. In addition, various cytokines and chemokines associated with inflammatory responses (CXCL6, CXCL12, CXCL16, IL-1β, IL-6, IL-34, M-CSF, TNF-α) have also been reported to be associated with tumor progression and hearing loss in sporadic VS [172]. Wu et al. showed in *NF2*-related schwannomatosis mouse models that losartan treatment improved hearing by blocking fibrotic and inflammatory signaling and normalizing the TME [179].

NF-κB is a key major mediator of inflammation and is a transcription factor that regulates numerous cellular processes, including cell proliferation, apoptosis, inflammation, and malignant transformation [180]. The *NF2* product, merlin, has been demonstrated to act as an inhibitor of NF-κB in mouse fibroblasts and rat glioma cells, and merlin has been shown to be an important negative regulator of NF-κB activity in human schwannoma cell lines [181–183].

Inflammation within the tumor microenvironment appears to be reflected in the systemic circulation. A systemic inflammatory response characterized by the leukocyte component of the peripheral blood, such as the peripheral blood neutrophil/lymphocyte ratio (NLR), may be associated with tumor growth [184, 185]. Moreover, Vasilijic et al. identified immune-related candidate plasma biomarkers associated with tumor size and hearing loss in sporadic VS as S100B and MCP-3, respectively [186].

Heterogeneity

Compared to NF2-associated schwannomas, sporadic schwannomas are monoclonally derived and genetically homogeneous [187]. However, they exhibit marked clinical and therapeutic heterogeneity, posing a significant challenge to successful treatment. The mechanisms underlying the diverse growth patterns and treatment responsiveness of schwannomas remain unclear. In understanding the intra- and inter-tumoral heterogeneity underlying the development and progression of schwannomas, scRNA-seq emerges as a valuable investigative approach.

The findings from scRNA-seq studies illustrate that schwannomas are heterogeneous tumors involving multiple cell types. Xu et al. showed the gene expression differences in Schwann cells and fibroblasts in scRNA-seq of three VSs [168]. Similarly, Yidian et al. noted heterogeneous gene expression profiles in their scRNA-seq analyses of three VSs [169]. Barrett et al. described both the transcriptional and epigenomic profile of the VS TME at single cell resolution by combining scRNA-seq data from 15 VSs with scATAC-seq data from 6 VSs [11]. These results substantiate the intra-tumoral heterogeneity of schwannomas. Moreover, Chiasson-MacKenzie et al. demonstrated in a mouse model that *NF2* mutant Schwann cells alter cell polarity via the ErbB pathway, suggesting a self-generating model of intra-tumoral heterogeneity [188]. On the other hand, Huo et al. performed scRNA-seq on seven VSs and two normal nerves and found heterogeneity between VS and normal nerves as well as between individual VS tumors [189]. Barrett et al. used single-cell multi-omic analysis data to classify schwannomas into two groups [11]. These studies support the inter-tumoral heterogeneity of schwannomas.

Molecular differences between sporadic and NF2-associated schwannomas

In both sporadic schwannomas and NF2-associated schwannomas, *NF2* mutations are crucial for tumorgenesis. *NF2*-related schwannomatosis is an autosomal dominant genetic disorder resulting from germline mutations in *NF2* [4, 93]. This condition exhibits wide phenotypic variability and nearly 100% penetrance by 60 years of age [190]. Germline mutations associated with *NF2*-related schwannomatosis are identified in more than 90% of familial cases, with patients either inheriting the mutation from an affected parental allele or acquiring it *de novo*. Nearly half of the patients with *NF2*-related schwannomatosis present with *de novo* mutations without any family history, while approximately 60% of patients with novel mutations display mosaic *NF2*-related schwannomatosis [191, 192].

Beyond sharing pathological features, sporadic schwannomas and NF2-associated schwannomas exhibit similar characteristics within the tumor microenvironment [164, 193, 194]. In previous reports, these tumors exhibited a high degree of similarity in inflammatory markers, signaling pathways, and gene expression profiles, with macrophages accounting for approximately one-third of the cell milieu in both forms [194]. Moreover, Lewis et al. analyzed 20 NF2-associated VSs and 24 size-matched sporadic VSs, demonstrating a correlation between microvascular and macrophage density. This led to the conclusion that inflammation is an important factor in the TME of VSs [164]. Thus, pronounced similarities have been observed in the TME between sporadic schwannomas and NF2-associated schwannomas. In contrast, NF2-associated meningiomas are reported to exhibit a marked immune response relative to sporadic *NF2*-mutant meningiomas, indicating that these TMEs are different in meningioma [195].

Animal models

A mouse schwannoma cell line SC4 was derived from schwannomas in P0-*Cre*; $Nf2^{flox/flox}$ mice [196, 197]. Gehlhausen et al. generated *Postn-Cre*; $Nf2^{flox/flox}$ mice, which develop multiple paraspinal schwannomas and VS, and examined the functional impairments in hearing and balance [198]. HEI-193, a human schwannoma cell line, was established from a patient with *NF2*-related schwannomatosis [199]. Xue et al. established an immortalized JEI-001 cell line derived from a human sporadic vestibular schwannoma patient with features distinct from those of HEI-193 cells [200].

One challenge with xenograft studies in schwannomas is the slow growth rate of the tumor. In contrast, a vestibular schwannoma xenograft zebrafish model has been reported to feature a relatively brief model-building period, with xenograft cells growing into tumor masses

two days after transplantation [201]. Furthermore, intracranial xenograft models have been reported in which the cell line is directly transplanted into the cochlear vestibular nerve at the cerebellopontine angle of mice [202–204]. These models are expected to be used in studies not only pertaining to tumor growth and hearing loss, but also the effects of drug therapy. Patient-derived xenograft (PDX) models are also essential for evaluating drug therapies. However, the attempts to create PDXs of schwannomas have been largely unsuccessful, and their validity in preclinical studies is limited [205, 206]. Recently, Zhao et al. reported the development of a PDX and cell line that exhibit characteristics similar to NF2-associated schwannoma [207]. Although it is challenging to establish a reproducible animal model of schwannoma, it remains a crucial step in the advancement of novel drug therapies, warranting further research in this area.

Conclusions

As this review indicates, there has been a notable advancement in the molecular underpinnings of schwannomas of the central nervous system. Although *NF2* mutations are the primary cause of schwannomas, not all tumors exhibit *NF2* mutations, suggesting that dysregulation of other genes may be involved in tumor pathogenesis. Furthermore, novel genetic alterations have been identified, including the *SH3PXD2A::HTRA1* fusion gene, *SOX10* indel mutation, and VGLL-fusions in schwannomas. Additionally, recent research efforts have furthered our comprehension of TME associated with schwannomas, gradually providing insights into the mechanisms underlying schwannoma development and tumor heterogeneity. However, the current state of knowledge regarding these molecular findings on schwannomas remains insufficient and controversial. As a future perspective, it will be crucial to not only further aggregate molecular biological findings, but also to integrate these to clinical research through translational studies. Such efforts hold the potential to facilitate the discovery of prognostic markers and the development of novel targeted therapies.

Abbreviations

CNS	Central nervous system
DEG	Differentially expression gene
LOH	Loss of heterozygosity
MPNST	Malignant peripheral nerve sheath tumor
NGS	Next-generation sequencing
PDGFR	Platelet-derived growth factor receptor
RT-PCR	Reverse transcription polymerase chain reaction
scRNA-seq	Single-cell RNA sequencing
TME	Tumor microenvironment
VEGF	Vascular endothelial growth factor receptor
VS	Vestibular schwannoma
WHO	World Health Organization

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