Clinical and biological behaviour of vestibular schwannomas: signalling cascades involved in vestibular schwannoma resemble molecular and cellular mechanisms of injury-induced Schwann cell dedifferentiation

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Abstract
Vestibular schwannoma (VS) is a slow-growing, intracranial extra-axial benign tumour that develops from the vestibular nerve, or very rarely from the cochlear nerve. The tumour is located along the course of the nerve that is between the inner ear and the brainstem in the internal auditory canal and the cerebellopontine angle. This review summarizes the current knowledge on clinical and molecular aspects of VS development with special emphasis on cellular dedifferentiation of Schwann cells to plastic, neural crest stem cell-like phenotype. We also summarize the signalling cascades potentially involved in VS development and dedifferentiation of Schwann cells after injury.

Introduction
Two types of vestibular schwannoma (VS) can be distinguished: sporadic VS account for 95% of all cases and develop spontaneously as unilateral lesions in the internal auditory canal (IAC) and cerebellopontine angle (CPA). The typical age distribution is between 40 and 60 years with no gender prevalence1. Bilateral lesions are pathognomonic for neurofibromatosis type 2 (NF-2). NF-2 is an autosomal dominant multiple neoplasia syndrome and one of the most common genetic disorders. It is characterized by bilateral VS, multiple meningomas, cranial nerve tumours, spinal tumours and eye. NF-2 is caused by mutations in the tumour suppressor gene NF2, and has an incidence of 1 in 25 000 newborn infants5. Half of the patients do not have a family history of the disease and therefore represent new germ-line mutations.

Although the inactivating mutation of the tumour suppressor gene NF2 and its gene product Merlin is widely believed to be a “common denominator” during development of VS, the involved signalling cascades are far from being deciphered. Moreover, although hyperproliferative Schwann cells are cellular origin of such benign tumours, a deep understanding of the involved molecular processes is missing so far.

Incidence and classification of VS
VS represent 8% of all intracranial tumours and 80% of all CPA tumours3,4. A US nationwide study determined the incidence of VS to be 5 cases per million per year between 1977 and 1981. Between 1992 and 1995, an incidence of 10 cases per million per year was published5. In 2004, Tos et al. estimated an incidence of 11.5 cases per million per year between 1976 and 20016. Concordantly, the US national tumour registry reported an overall VS incidence rate of 1.1 cases per 100,000 per year7. Looking into the different aetiologies, Evans et al. found an incidence of 1 case in 80 000 individuals for sporadic VS and 1 case in 70 000 if NF2-related tumours were included4,7. The true prevalence of VS if asymptomatic tumours are included is likely to be significantly greater (7/10 000)8.

Based on magnetic resonance imaging (MRI) findings and clinical symptoms, Selesnick et al. proposed a classification with four tumour stages6:

- Intracanalicular
- Cisternal
- Brainstem compressive
- Hydrocephalic

The stages imply different symptoms of increasing severity and specificity. The symptoms in the early stage of the disease are unspecific and include progressive, high-frequency unilateral hearing loss in majority of the cases (80%), often combined with tinnitus3. Compression and stretching of the nerves of the IAC caused by the expanding lesion are postulated to be the reason for this gradual degradation.

However, 25% patients present with sudden deafness due to occlusion of the labyrinthine artery or rather its branches (cochlear, vestibular and vestibulocochlear arteries)10. More than 50% patients complain of balance problems. In early stages of the disease, vertigo is the more common symptom, whereas patients with larger tumours more often complain of disequilibrium8,11. Vertigo, an illusion of
motion, is reported in 19% patients with tumours <1 cm. The incidence of disequilibrium, a sense of floating or unsteadiness, is reported to be proportional to the size of the tumour, occurring in 48% patients. These findings indicate that vertigo is present in early disease, whereas disequilibrium is a later symptom. Other typical symptoms occur in later stages and may include headache (19%–85%), trigeminal nerve dysfunction (approx. 20%), facial nerve dysfunction (10%–18%) and increased intracranial pressure (nowadays very rare). Brainstem and cerebellar symptoms as well as lower cranial nerve dysfunctions, such as dysarthria, hoarseness, aspiration or dysphagia, are common in the literature. In the modern era, they are rarely associated with isolated VS. These symptoms hint more towards jugular for men tumours. Bilateral VSs, pathognomonic for NF2, are more aggressive, showing rapid growth, greater bone erosion and severe affection of nerves and vessels in the IAC and the CPA. Two different phenotypes have been described, the severe (Wishart) phenotype and the mild (Gardner) phenotype, with distinctly different prognoses.

Most recent studies observe slow growth rates of VSs ranging from 0.1 to 0.23 cm/year. Less than 30% of untreated VSs exhibit growth rates greater than 0.2 cm/year, whereas tumours larger than 2.0 cm are statistically more likely to grow fast. Overall, three different growth patterns can be distinguished: no or very slow growth, slow growth (approx. 0.2 cm/year) and fast growth (>1.0 cm/year).

**Current diagnosis and treatment options**

As mentioned earlier, the typical symptoms of VS are unspecific. On presentation of the conveying symptoms, a systematic otologic and neurologic assessment, as well as pure tone and speech audiometry is essential for proper diagnosis. A typical finding in VS is the ipsilateral reduction of speech discrimination, which is out of proportion to pure tone threshold responses. However, the specificity of these subjective tests is low.

Auditory brainstem response (ABR) testing is easy to record and independent of the patients' cooperation. ABR, also known as brainstem electric response audiometry involves the recording of synchronized action potentials of auditory neurons evoked by a distinct click stimulus. The action potentials are displayed as distinct peaks (waves) representing different parts of the auditory nerve and tract. Waves I and V are the most consistent waves and therefore used for latency measurements. For diagnosis of retrocochlear pathologies, the latencies between wave I and V are compared in both ears. A latency difference of greater than 0.2 milliseconds is considered abnormal and hints towards VS. Since ABR is not affected by sedation or anaesthesia, it can also be used for intraoperative nerve monitoring.

Since all other diagnostic tools are not very sensitive in detecting VSs, imaging is the key to diagnosis. Since its introduction in the 1980s, MRI is the gold standard. VSs appear hypo- to isointense in both T1- and T2-weighted images with strong enhancement of Gadolinium. T2-weighted images best demonstrate the displacement of cerebrosplian fluid by the tumour in the CPA. Despite being relatively nonhazardous even if Gadolinium is administered, MRI has a few obvious disadvantages: if metallic of even magnetic implants are present, it might be impossible to conduct MRI or there might be pronounced artefacts in the images. Besides, patient claustrophobia in the tight MRI scanners is not uncommon and open units are not comprehensively available (Figure 1).

In cases with contraindications for MRI, contrast-enhanced computed tomography (CT) is an option, although the sensitivity of CT is lower especially in smaller lesions. Of course, radiation exposure has to be taken into consideration too.

Generally, in times of cost pressure in the medical systems, imaging should be conducted if there is reasonable suspicion of VS, for example, if ABR latencies are pathologic.

Since there are several fundamentally different treatment options, counselling of patients with VSs is very important. Patient history and actual complaints have to be taken into consideration as well as age and general health of the patients. Tumour size and location play an important role and also patient preference should influence the decision towards one of the different treatment options. Treatment can follow three generally different paths: observation, microsurgery or irradiation. The main goals of every treatment should be tumour control with minimal deterioration of quality of life. Counselling of patients and decision making should have these two goals in focus. However, this is not the main focus of this paper.

Counselling and management of patients with NF2 poses essentially different problems and must be discussed separately.

Observation (“wait and scan”, or “watchful waiting”) is the treatment strategy of choice in small tumours without brainstem contact and little or no symptoms. Since nowadays, majority of the tumours are diagnosed in early stages (intracanalicular or cisternal), this treatment option is employed in most first time diagnosed VSs. Clinical and audiological re-evaluations on a quarterly or half-year basis are recommended. MRI should be repeated yearly for the first two years after diagnosis and after at least every two years later. In case of tumour growth or the occurrence or significant worsening of symptoms or if the patient wishes so, the treatment has to be reconsidered. This scenario is seen in about one-third of the patients, while in two-thirds of the patients, no intervention is necessary.

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The growth rates of VSs are very heterogeneous. Average tumour growth rates of between 0.3 and 1.42 mm per year have been published\textsuperscript{15}, but individual VSs might grow significantly faster.

Microsurgery is indicated for large tumours with brain stem contact or even compression, tumours with growth rates greater than or equal to 3 mm/yr or increasing cochleoves-tibular symptoms (disabling vertigo, hearing deterioration). Patient's consent has a significant impact on treatment options. In the recent literature, complete tumour resection could be achieved in 96\%–99\% with recurrence rates of 0.8\%–3\%\textsuperscript{15}. The rates of functional preservation of the facial nerve and, even more critical, hearing preservation are dependent on the size of the tumour; in tumours smaller than 2 cm\textsuperscript{3}, functional preservation of the facial nerve (House Brackmann grade I and II) is reported in 96\% and hearing preservation (Gardner-Robertson Class I and II) was achieved in 48\%. In tumours of 2 to 4 cm\textsuperscript{3}, facial nerve preservation was 74\% and hearing preservation was 25\% and in larger tumours, facial nerve preservation dropped to 38\% and hearing preservation was 0\%\textsuperscript{15}.

The three different approaches for microsurgery imply distinct assets and drawbacks. Significant differences in functional nerve preservation do not exist between different approaches. The translabyrinthine approach sacrifices any residual hearing and is thus mostly applied when hearing preservation is not an issue. However, the a particular advantage of this approach is that it is the most direct route to the CPA; it allows for the visualization of the IAC in its entirety while all drilling is extradural, and no brain tissues have to be displaced\textsuperscript{16}. Thus, it allows for the removal of tumours of all sizes. Furthermore, the facial nerve can be exposed—if necessary—from the brainstem to the stylomastoid foramen.

The retrosigmoid or lateral suboc-cipital approach is the most frequently used approach for microsurgical VS removal. It allows for the resection of tumours of all sizes and it also allows for hearing preservation\textsuperscript{17}. As mentioned earlier, the rates of hearing preservation as well as functional facial nerve preservation depend on the size of the tumour. The cerebellum has to be medialized to visualize the CPA.

The middle fossa (trans temporal) approach is suitable for small intra-canaliculă tumours extending <1 cm into the CPA without brainstem contact. The approach is not recommended for the removal of larger tumours. Serviceable hearing can be preserved reportedly in around 60\% of the cases\textsuperscript{18}. However, the temporal lobe needs to be elevated which bears the risk of complications especially in patients older than 69 years.

The third treatment option in VS is irradiation. Two techniques with different characteristics are available: stereotactic radiosurgery (SRS) and stereotactic radiotherapy (SRT). SRS is usually performed in 1–5 sessions and uses conformal radiation dose plans. The tumour margin dose is usually 12–14 Gy (up to 25 Gy in multisession SRS), maintaining a high intratumoural dose with a steep radiation fall-off to preserve adjacent tissues\textsuperscript{19}.

SRT uses higher maximum doses (usually 54–60 Gy) and achieves safety by dose fractionation. The desired dose is delivered in 30 to 33 fractions of 1.8 to 2.0 Gy\textsuperscript{19}.

**Figure 1:** (a) MRI, cranial section, right-sided vestibular schwannoma, slightly infiltrating the brain stem, a 72-year-old Caucasian female patient with moderate-to-severe retrocochlear hearing loss. (b) Macroscopic appearance of the VS in situ shown in (a). Size is 1.8 x 2.2 x 1.64 cm.
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This plasticity was demonstrated by their ability to dedifferentiate into a more primitive phenotype after injury in vivo or in different experimental approaches in vitro. Such phenotypic instability has been postulated to be a direct consequence of a close developmental relationship of SCs with early neural crest cells. Moreover, different recently described adult neural crest-derived stem cell populations seem to be directly Schwann cell-related.

Importantly, VSs evolve from the abnormal growth and proliferation of Schwann cells, at their junction with glial cells surrounding the vestibular nerve. In contrast to cellularly heterogeneous peripheral nerve sheath tumours like neurofibromatosis type 1, VS consist nearly exclusively of hyperproliferative Schwann cells and supporting vasculature (reviewed in Ref.21). In this context, it is noteworthy that in contrast to mature Schwann cells, VS Schwann cells express, in addition to S100b and nestin, high levels of the neurotrophin receptor p75, whose expression is a hallmark of neural crest cells as well as immature Schwann cells (Figure 2). Thus, one may hypothesize that the development of VS may be tightly linked with deregulated cellular dedifferentiation of post-mitotic Schwann cells to a neural crest-like phenotype. Indeed, a recent study suggested that at least after treatment with platelet-derived growth factor (PDGF), VS Schwann cells display neural crest stem cell characteristics such as the expression of Oct4 and Nanog as well as the ability to form neurospheres. Here we discuss molecular signalling cascades involved in the pathogenesis of VS and highlight a potential overlap with mechanisms regulating the dedifferentiation of Schwann cells.

**Signalling pathways related to VS-pathogenesis**

In contrast to several tumours of the central nervous system, no obviously common amplifications of

**Schwann cells are highly plastic, neural crest-derived cells and represent the cellular origin of VS**

During the vertebrate development, the so-called embryonic neural crest cells migrate out of their niche between the newly formed ectoderm and neural tube, generating not only several mesenchymal cell types including muscle cells or craniofacial bones but also cells of the peripheral nervous system (PNS) such as Schwann cells (SCs; reviewed in Ref.22). SCs, first described by Theodor Schwann as cells ensheathing peripheral nerves, are the most plastic, postmitotic cell type within the adult body.

Remarkably, although it is small, a definite risk of malignant transformation exists after irradiation.

**Figure 2:** Immunohistochemical analysis of typical VS-tissue. VS tissue samples were cryosectioned, fixed and subsequently stained with antibodies raised against the adult Schwann cell marker S100β and nestin and the immature Schwann cell/Nerve crest marker p75. Note the high expression of S100β adjacent to the nerve fibre (N). Beside the Schwann cell-specific marker S100β (upper panel, red) and nestin (lower panel, red), VS Schwann cells also express high levels of the immature Schwann cell and neural crest marker p75.
tumour-related genes were found in VS, suggesting a complex molecular mechanism of its development. However, a vast majority of VSs are often associated with a biallelic inactivating mutation of NF-2 and its gene product Merlin (Schwannomin) on chromosome 22q12.4. Merlin is a 70 kDa protein belonging to the ezrin/radixin/moesin family (“ERM-proteins”) lacking the actin-binding domain characteristic for the other family members. Merlin is highly expressed in terminally differentiated cells including Schwann cells, but absent or expressed at dramatically low levels in premigratory neural crest stem cells and early Schwann cell progenitors.

Its cellular action is mainly anti-proliferative and seems to occur at least partially due to inhibition of NF-kB and its target gene cyclin D1. Although the message for Merlin can usually be detected, the Merlin protein is not present in VS. Schulze and colleagues demonstrated in 2002 that transduction of wild-type Merlin into schwannoma cells resulted in significantly reduced proliferation and increased proportion of apoptotic cells. Besides the regulation of NF-kB signalling, Merlin seems to be involved in signalling pathways activated via growth factors such as epidermal growth factor (EGF), insulin-like growth factor 2 (IGF-2), PDGF and fibroblast growth factor (FGF-2). Consequently, the downstream signalling cascades such as Ras/Raf/MEK1/2/ERK, PI3-K/AKT, p38 MAPK as well as JNK are highly active in VS and have tremendous impact on the proliferation and resistance to apoptosis. In addition to the growth factors, there is evidence suggesting the involvement of neurotrophic factors such as nerve growth factor (NGF) and the respective signalling pathways. In this context, it has been described that VS patients show significantly elevated levels of circulating NGF. According to this study, Bonetti et al. reported high expression of the NGF-receptor p75 in VS Schwann cells, which is absent in healthy, postmitotic Schwann cells. Moreover, healthy, non-VS-Schwann cells lack not only p75 but also the ligand NGF. Interestingly, in schwannoma cells, NGF was described to induce NF-kB activity and the JNK-pathway selectively via p75 and not via the high affinity receptor TrkA.

Schwann cell dedifferentiation pathways resemble signalling cascades associated with VS development

Signalling cascades, which regulate the dedifferentiation of Schwann cells into a stem cell or at least progenitor state, seem to greatly resemble mechanisms involved in the pathogenesis of VS.

In 2006, Jungnicken and co-workers were able to demonstrate that mice overexpressing the potent Schwann cell growth factor FGF-2 regenerate sciatic nerve injury faster than wild-type animals, suggesting a role of this growth factor during regeneration and dedifferentiation. Besides FGF-2, EGF also seems to play an important role in regeneration after injury. In 1992, Toma and colleagues observed increased levels of the EGF receptor in Schwann cells after nerve injury, which was inversely proportional to the expression of the mature marker P0. In accordance with these findings, we recently dedifferentiated adult sciatic nerve Schwann cells in vitro to neural crest-like phenotype by serial explantation followed by culture in the presence of FGF-2 and EGF. Further, VS-related growth factors IGF-1 and -2 act as autocrine growth factors and are able to induce proliferation of myelinating and non-myelinating Schwann cells.

Similar to growth factors, neurotrophins such as NGF and their receptors are also known to influence Schwann cells during nerve regeneration as well as during VS development and progression. Using lineage tracing experiments, Nagoshi and co-workers demonstrated that experimental injury leads to dedifferentiation of adult p75-negative Schwann cells to plastic cells which rapidly upregulate p75, followed by proliferation and finally by redifferentiation to adult phenotype 65 days after injury. Similar to p75, the NF-kB-subunit p65 is not expressed in adult, healthy Schwann cells. However, after injury-induced dedifferentiation of Schwann cells, a dramatic upregulation of NF-kB p65 expression was observed 12 h after injury which was accompanied by increased DNA binding. In addition to NF-kB and p75, JNK/c-Jun signalling also appears to be crucially involved in the phenotypic switch from adult to the stem cell-like state. This fact is underlined by the significantly reduced dedifferentiation capability of Schwann cells in c-Jun-deficient mice, which results in a significantly diminished ability to endogenously regenerate injured nerves. Interestingly, also c-Jun is not expressed in healthy adult Schwann cells and can only be detected in the context of regeneration and dedifferentiation.

A further crucial signalling cascade involved in both pathogenesis of VS and dedifferentiation of Schwann cells seems to be the Ras/Raf/MEK/ERK pathway. It has been demonstrated that Schwann cells infected with retroviruses expressing oncogenic Ras LXSN rapidly downregulate the mature marker P0, which is accompanied by upregulation of cyclin D1 and phosphorylation of the downstream kinase ERK. Further evidence for involvement of this pathway was recently provided by Napoli and co-workers. In this study, the authors used a transgenic mouse model, in which tamoxifen-inducible Raf (Ras-inducible downstream kinase) is expressed under the control of Schwann cell-specific promoter P0. Remarkably, treatment with tamoxifen was sufficient to dramatically increase the percentage of p75-expressing, immature cells within the
nerves, suggesting rapid dedifferentiation of Schwann cells.

As mentioned earlier, the p38 MAPK cascade also potentially takes part in the development or progression of VS. Also, in this case, an obvious correspondence with signalling in dedifferentiation was reported. In this respect, signalling via p38 MAPK was recently reported to prevent the redifferentiation of dedifferentiated Schwann cells. In addition, the authors reported that forced ectopic activation of p38 MAPK is sufficient to drive differentiated Schwann cells to an immature Schwann cell phenotype.

Altogether, the signal molecules and resulting signalling cascades involved in VS pathogenesis and dedifferentiation show an obvious overlap (Figure 3). A huge cross-coupling of the respective signalling cascades was reported in other systems and should be investigated in VS in more detail.

Discussion and conclusion

VS are direct progeny of adult Schwann cells, which may have re-entered the cell cycle and dedifferentiated into a more primitive, neural crest-like phenotype. After injury, such dedifferentiation of Schwann cells functions as a regenerative process and proceeds through three distinct phases: dedifferentiation including demyelination, re-entry into the cell cycle and proliferation and finally redifferentiation into the cell types that require regeneration. During VS development, such processes may be induced without damage by the lack of negative control by Merlin resulting in hyperproliferation without subsequent redifferentiation. The molecular mechanisms involved in both processes—dedifferentiation in regenerative context and during VS-pathogenesis—show a great overlap including growth factors and neurotrophins as well as the downstream effector signalling cascades.

Figure 3: Summary of signalling cascades potentially involved in VS development and dedifferentiation of Schwann cells after injury. Bold red lines represent pathways experimentally described in both systems, whereas black lines represent potential cross-couplings. Note that at least p75, NF-κB p65 and c-Jun are not expressed in healthy, mature Schwann cells, but are present/active in VS and during injury-induced regeneration.
providing potential novel intervention points for therapy approaches. Such therapeutic intervention may include a specific blockade of the proliferation of dedifferentiated Schwann cells into VS or exogenously induced redifferentiation of VS Schwann cells into the post-mitotic, non-proliferative phenotype.

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