Tumor biological characteristics of Vestibular Schwannoma

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CHAPTER 1

Introduction and outline of the thesis

Introduction

Vestibular schwannoma (VS) is a benign neoplasm recapitulating the differentiation repertoire of the myelin-forming Schwann cells forming the nerve sheath that insulates the vestibular portion of the eighth cranial nerve. Other more archaic names used to designate this tumor are acoustic neuroma, acoustic neurinoma or vestibular neuroma. Given its origin and biological composition, vestibular schwannoma is the most appropriate terminology[1,2]. Though benign these tumors are not harmless, their location in the internal auditory meatus and cerebellopontine angle can cause severe morbidity and in rare cases may even lead to death by brainstem compression when they are left untreated.

VS account for approximately 80% of all neoplastic lesions found in the cerebellopontine angle (CPA) [3]. Approximately 90% of all VS are unilateral sporadic tumors [4]. In rare cases these tumors occur bilateral which is a pathognomonic symptom for the hereditary disease neurofibromatosis type 2 (NF2). Recent years the number of diagnosed VS is rising. This rise is largely a result of the increased application of magnetic resonance imaging (MRI) scanning which for example leads to the detection of more asymptomatic lesions. Next to its widespread use the continually improving sensitivity of MRI is an important contributor to this phenomenon as well. The current incidence rate of VS lies around 20 per million people per year and this number is still increasing[5-8].

The biological background of schwannomas is still not fully understood. An important step in unravelling the genetic basis of VS formation was made by Rouleau, Seizinger and Trofatter who isolated the neurofibromatosis type-2 gene (*NF2*) during the late 80's of the twentieth century [9-12]. The *NF2* gene is located on chromosome 22q11 and it encodes for the tumor suppressor protein merlin [13]. Heterozygous germline inactivating mutations affecting *NF2* cause neurofibromatosis type-2 while biallelic somatic mutations of *NF2* are found in sporadic, unilateral vestibular schwannomas. [14]

This thesis mainly comprises research that is performed on sporadic vestibular schwannomas. NF2-related and sporadic vestibular schwannomas show considerable differences in their clinical presentation and they often require different and more complex therapeutic approaches. At the same time there are quite some similarities between both tumors and biological or clinical knowledge about one type can be beneficial to the understanding of tumorgenesis of the other type as well.

Unilateral vestibular schwannomas usually occur in adult patients with a peak incidence ranging from forty to sixty years [4,15] Main clinical symptoms are unilateral sensorineural hearing loss, tinnitus and balance disorders. In rare cases larger tumors can cause unsteadiness, trigeminus neuralgia, facial nerve paralysis and long tracks symptoms. If untreated, progressive tumors can finally lead to brain stem compression, obstructive hydrocephalus and increased intracranial pressure.

During the 20th century the tools and techniques to detect vestibular schwannomas have greatly improved from the tunic fork to audiometry and vestibular testing, auditory brain stem evoked response testing, computed tomography and finally magnetic resonance imaging (MRI)[16]. In 1987 gadolinium enhanced MRI was introduced, this technique has become the gold standard for the diagnosis of VS [17]. Tumors as small as 3 mm can now be detected [18]. The ability to detect VS in an ever-earlier phase has led to an increasingly complex decision-making regarding the best way to deal with these tumors once they are diagnosed.

Therapeutic management

Current therapeutic management of sporadic VS comprises three main options: conservative management by observation with scheduled MRI follow-up (wait-and-scan), microsurgery or radiotherapy. Although scarcely applied and not part of standard therapy yet, pharmacotherapeutic options are emerging as well. Choosing the most suitable therapy is mainly based on symptoms, radiological assessment and patient's preference.

Observation

Because of the increase in use and quality of MRI more and more VS are diagnosed in early tumor stages, often without even causing symptoms. Given the fact that VS are relatively slow growing tumors with an average growth rate of approximately 1-3 mm/year [19-21] the initial treatment strategy in these cases will be observation. This strategy was first suggested for the elderly or otherwise frail patients. Because natural history studies have indicated that up to 50% of VS show no significant growth in the first five years after diagnosis [19] this strategy nowadays is applied to younger patients as well. The goal of this strategy is to delay or even avert treatment related morbidity. The main drawback of this policy is that in case of rapid tumor progression the complexity of active treatment will have increased compared to the conditions at initial diagnosis. One of the hallmarks of VS is their variable growth pattern. This makes prediction of future tumor behavior difficult and even a strict wait and scan protocol with systematic radiological follow-up can sometimes result in unexpected and unfavorable situations. Better understanding of the biological processes behind VS growth can help to improve prediction of tumor behavior in order to single out potentially aggressive tumors at an early stage.

Microsurgery

First successful attempts to surgically remove VS were performed over a century ago. Unfortunately, these pioneering surgeons accomplished success in a minority of cases. Shortly before the start of World War One mortality rates were approximately 80% and almost all patients suffered major complications [22]. Over the twentieth century surgical outcomes have greatly improved. A large contribution to this growing success rate was the application of the translabyrinth microsurgical approach introduced by the otologist House

in 1963. Current reviews indicate that mortality associated with VS surgery is rare and occurs in less than 1% of cases [23]. Microsurgical techniques provide good tumor control but there is still an undeniable risk of iatrogenic complications such as damage to adjacent cranial nerves, cerebrospinal fluid leak, intracranial hemorrhage and meningitis. So far, microsurgery is the only treatment strategy to achieve (near total) tumor removal while radiotherapeutic approaches merely prevent additional tumor growth. Surgery has a high efficacy and less than 1% of patients require additional treatment after incomplete tumor resection[23]. Because of the significant risks associated with microsurgery it is important that patients and physicians make a balanced decision in which benefits have to be weighed against potential complications. Main indications to apply microsurgery should be tumors that progress rapidly or large tumors (>30mm) showing radiological or clinical signs of brainstem compression.

Radiotherapy

The Swedish neurosurgeon Lars Leksell introduced the concept of stereotactic radiosurgery (SRS) for brain tumors in 1951. The first time this technique, also known as the Gamma Knife, was applied to a VS was in 1969 [24]. Initially high-dose treatment protocols were used. This led to good tumor control but also had a significant effect on hearing loss and rates of facial and trigeminal neuropathy were high. Over the years the dose threshold has been lowered to 12 or even 11 Gy. At these levels permanent facial palsy is less than 1% and over 90% of patients show durable tumor control[25]. Recent years another type of particle therapy, proton therapy, is increasingly used. This technique uses protons instead of photons and has the advantage of further reducing the radiation dose to surrounding tissues, hypothetically leading to less iatrogenic damage. It used to be a scarce treatment modality but the availability has increased tremendously during the past decades. A recent article reviewing proton therapy indicated that data on its application in VS treatment is scarce and the few reports that are present do not show a favorable outcome in comparison to conventional radiotherapy[26,27]. The role of proton therapy in the treatment of VS remains unclear and additional research is needed to properly verify its effect before making it part of standard treatment options.

Pharmacotherapy

Despite the improvements of surgical and radiotherapeutical techniques these modalities will always carry a certain risk of inflicting iatrogenic damage to surrounding structures. Pharmacotherapeutic treatment might be an additional treatment strategy with less iatrogenic effects or complications. It could also benefit patients that are not eligible to undergo current forms of therapy.

Treating sporadic VS with drugs is not part of standard clinical practice yet. Nevertheless there are promising reports on the effect of antiangiogenic treatments for patients suffering

from NF2 related vestibular schwannomas. The most extensively tested drug in the context of VS is bevacizumab. This is an U.S. Food and Drug Administration approved anti-VEGF antibody used for the treatment of several types of cancer. A number of reports demonstrated stabilization and even tumor shrinkage in a significant amount of patients [28] [29,30]. Other forms of targeted therapy showed encouraging results as well [31] but there are also reports on drug resistant tumors[32]. **Chapter two** provides a more extensive overview of the current knowledge on-, and the future prospectives of pharmacologic therapies and the tumor biological processes behind them. The genetic- and micro environmental factors that determine vestibular schwannoma genesis and development will be discussed in **chapter two** as well.

Choice of therapy

Choosing the most suitable therapy for individual vestibular schwannoma patients can be a complex matter. In the obvious cases, such as fragile patients with small and stable intracanalicular tumors or young patients with progressing large brainstem compressing tumors, the choice can be quite clear. However, most patients find themselves between these extreme ends of the spectrum. For this category of patients there is the possibility to choose between different treatment modalities. This choice has to be made by the patient together with his or her physician. The fact that even among experts there remains controversy when it comes to selecting the correct therapy makes shared-decision-making even more difficult. During the past decades a vast amount of research into this topic has been performed but so far a balances consensus has yet to be reached.

The fact that all modalities have different goals makes a correct comparison between them difficult. For microsurgery an important goal is total tumor removal, for radiosurgery stopping tumor progression is the main outcome to measure success while the desired result of the wait and scan policy is avoiding disproportional treatment related morbidity. Before reaching consensus about treatment there should be more consensus about the goals and criteria by which success can be defined. A more general method to assess the effect of treatment is to determine quality of life (QoL). The PhD theses of Godefroy in 2010 and van Leeuwen in 2016, both at the Leiden University, indicated the importance of this measuring instrument. QoL assessments can help to increase the comparability of the therapeutic modalities, this will benefit the research that is performed to refine the decision-making with regard to the correct timing and method of treatment. Another factor that complicates the controversy regarding the choice of therapy is the unpredictable behavior of VS. As mentioned earlier, for the cases at both ends of the phenotypic spectrum the required treatment strategy can be obvious. Unfortunately in a large proportion of patients, often the ones with medium sized tumors, it is difficult to make a good prognosis of future tumor growth. The controversy over the choice of therapy is most evident in this category of patients.

Increased accuracy of predicting tumor growth will help to narrow down the group of patients for whom it is unclear which therapy will benefit them the most. So far the only proven

prognostic factors of tumor growth are observed growth during follow-up and cystic degeneration [21,33]. More insight into the tumor biology of VS improves the understanding of its growth pattern, which in turn benefits the search for better prognostic factors of tumor growth. A recent example of research with a similar purpose was the identification of loss of H3K27 tri-methylation as a prognostic marker for malignant peripheral nerve sheath tumors[34]. Next to improving the accuracy of predicting tumor progression, tumor biological research has the goal of identifying potential targets for pharmacotherapy. The current thesis can be seen in this context.

Aims and outline of the thesis

In this thesis a number of tumor biological characteristics of sporadic VS are analyzed. We mainly focus on prognostic markers which could benefit individual decision making and processes that can form a potential target for therapy. Prior tumor biological research on VS has predominantly been performed on neurofibromatosis type 2 related tumors, sporadic tumors have been studied to a much lesser extent. **Chapter 2** provides an overview of the tumor biological research that has been performed on both types of VS but it focusses on the sporadic tumors. Current knowledge about the genetic profile of VS is described and the roles of angiogenesis and the microenvironment on tumor progression are discussed. It also comprises a summary of targeted therapy that has been applied so far.

Chapter 3 presents an immunohistochemical analysis of the presence of markers for cell proliferation (Histone H3 and Ki-67), vascularization (CD31), inflammation (CD45 and CD68) and intratumoral bleeding (hemosiderin). The expression of these markers was correlated to clinical parameters such as tumor size, patient age at time of surgery and duration of symptoms.

Chapter 4 describes an allele specific quantitative real-time PCR assay that was performed on tumor specimens of forty-eight patients in order to detect the presence of the thirteen most frequent mutations affecting *BRAF, EGFR, PIK3CA,* and *KRAS*. These genes encode for proteins that are members of the MAPK/ERK cell signaling pathway which is associated with uncontrolled cell growth. This pathway is known to be up regulated in VS and *BRAF* mutations have already been found in other sporadic non-head and neck schwannomas. If present, mutated *BRAF* can function as a potential target for therapy.

In **chapter 5** the inflammatory microenvironment of vestibular schwannomas is investigated by analyzing the presence of tumor associated macrophages (TAM). These macrophages are known to support tumor progression by stimulating processes like angiogenesis. The presence of TAM is analyzed by immunofluorescent staining for CD163. The level of CD163 expression is assessed and compared with the degree of angiogenesis and tumor growth in 20 sporadic vestibular schwannomas.

In **chapter 6** the expression of the breast cancer resistance protein BCRP is studied in a selection of peripheral nerve sheath tumors i.e. vestibular schwannomas, plexiform neurofibromas and malignant peripheral nerve sheath tumors (MPNSTs). All three tumor subtypes are known to display a certain degree of intrinsic resistance to drug therapy. BCRP is a transmembrane efflux transporter associated with drug resistance in various types of cancer and it is also part of the blood-brain-barrier. Targeting BCRP can enhance drug susceptibility of neoplastic tissues. In order to investigate the role of this protein in the biology of peripheral nerve sheath tumors an immunohistochemical staining for BCRP is performed on a tissue microarray. This array comprises 22 sporadic vestibular schwannomas, 10 plexiform neurofibromas and 18 MPNSTs.

Chapter 7 elaborates on the inflammatory microenvironment by investigating the macrophage colony stimulating factor (M-CSF) and interleukin-34 (IL-34). These two cytokines play a key role in the recruitment of TAMs and thereby form a potential therapeutic target. An immunohistochemical analysis of these proteins is performed in the same patient cohort as described in chapter 4. The presence of M-CSF and IL-34 will be related to the presence of TAMs, the degree of angiogenesis and volumetric tumor growth. Finally the results of the thesis are summarized and discussed in **chapter 8**, and future perspectives for research are indicated.

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