Tumor biological characteristics of Vestibular Schwannoma

Vries, M. de

CHAPTER 8

Summary and concluding remarks

Vestibular schwannomas are benign tumors recapitulating the differentiation repertoire of the Schwann cells that are part of the nerve sheath that insulates the vestibular portion of the eighth cranial nerve. These tumors classically arise within the internal auditory canal and usually progress into the cerebellopontine angle. One of the clinical hallmarks of these tumors is their capricious growth pattern. A significant portion of tumors are limited in size and remain indolent after they have been diagnosed. In contrast to this relatively harmless phenotype there are also tumors with a faster and more extensive growth pattern that are capable of causing brainstem compression or paralysis of adjacent cranial nerves[1]. This unpredictable behavior is one of the main clinical problems in the optimal management of vestibular schwannomas. Current treatment comprises observation by sequential MRI scanning for smaller and indolent tumors. For larger tumors, tumors that display rapid growth or in case of major symptoms, such as invalidating vertigo, active treatment in the form of radiotherapy or microsurgery is indicated. Pharmacotherapeutic alternatives are emerging but they are not part of standard treatment yet.
The different studies on which this thesis is based have the general aim to increase current insights into the intratumoral biological dynamics associated with vestibular schwannoma progression. Enlarging our tumor biological knowledge helps to interpret tumor growth patterns which in turn is paramount to the search for prognostic markers and targets for therapy.

Chapter one serves as a general introduction to vestibular schwannomas with regard to their aetiology, symptoms and the diagnostic tools that are used for their detection. This chapter also provides an overview of current therapeutic management and it discusses the clinical dilemmas involved in the treatment of these tumors that have resulted in the research questions which form the basis of this thesis.

Chapter two is a review of the literature on the tumor biology of vestibular schwannomas. It describes current knowledge on the genetic profile of these tumors and provides an overview of the cell signalling pathways associated with vestibular schwannoma progression. The role of angiogenesis as an important factor in tumor growth is discussed, as well as its potential function as a target for therapy. Other targets for therapy are also summarized and potential subjects of future research are briefly addressed.

Chapter three investigates a selection of intratumoral processes that take place within vestibular schwannomas in order to identify biological factors that contribute to tumor progression. Tumor specimens of sixty-seven vestibular schwannoma patients were immunohistochemically analysed for cell proliferation (Ki-67, Histone-H3), neovascularisation (CD31) , macrophage expression (CD68) and the presence of intratumoral bleeding (hemosiderin). In this study we find no association between cell proliferation and clinical

characteristics of tumor growth. On the other hand, factors like neovascularisation and macrophage expression do seem to be related to tumor size and the tumor growth index. Additionally we find that intratumoral bleeding occurs to a higher degree in tumors that show cystic degeneration, which in turn is a known process contributing to tumor expansion[1]. We therefore hypothesize that growth of vestibular schwannomas is not based on cell proliferation alone, factors like degenerative changes, angiogenesis and inflammation influence progressive tumor growth as well.

Chapter four describes a gene expression assay for the thirteen most frequent mutations affecting *BRAF, EGFR, PIK3CA* and *KRAS.* The products of these genes are members of the MAPK/ERK cell signalling pathway. Increased activity of this pathway is associated with progression of different types of tumors including vestibular schwannomas[2,3]. This knowledge combined with the finding of *BRAF* mutations in a number of sporadic non head and neck schwannomas [4] lead us to investigate the hypothesis that vestibular schwannoma progression is influenced by the occurrence of accessory oncogenic mutations related to the MAPK/ERK pathway. We performed a gene expression assay in a selection of forty-eight vestibular schwannomas, all of which turned out to be negative for any of the mutations that were tested. This finding therefore doesn't support the hypothesis that vestibular schwannoma progression is accelerated by additional oncogenic mutations that arise during the development of these tumors.

Chapter five is a study that further investigates the role of inflammation within vestibular schwannomas. As described in chapter three, some vestibular schwannomas contain large amounts of macrophages. During the past decades it has become clear that in various types of neoplasms the inflammatory microenvironment has many tumor promoting effects[5]. Macrophages are one of the major determinants of this microenvironment[6]. These so called tumor associated macrophages can roughly be divided into two groups consisting of the classically activated M1 type macrophages versus the alternatively activated M2 type macrophages. Of these two categories the M2 type macrophages are the ones with the tumor promoting characteristics such as stimulating angiogenesis and dampening of the antitumor immune response[7]. Using immunofluorescent stains against CD163, a specific marker for M2 type macrophages, we determined the expression of M2 macrophages in tumor samples of twenty retrospectively analysed vestibular schwannoma patients. We also determined the degree of angiogenesis within these tumors. The results of this study indicate that some vestibular schwannomas contain large amounts of M2 type macrophages and additionally there seems to be a positive relation between the expression of these macrophages and angiogenesis as well as tumor growth rate. These results imply that tumor infiltrating M2 type macrophages may stimulate the progression of vestibular schwannomas, which in turn makes them a potential target for therapy.

Chapter six describes a study that investigates BCRP expression as a potential cause of drug resistance in a selection of peripheral nerve sheath tumors. BCRP is a transmembrane efflux transporter protein that seems to play an important role in different biological barriers such as the blood-brain-barrier[8,9]. Its presence has also been associated with drug resistance in cancer[10]. An immunohistochemical staining for BCRP was performed on a tissue microarray composed out of twenty-two sporadic vestibular schwannomas, ten plexiform neurofibromas and eighteen malignant peripheral nerve sheath tumors. The findings of this study demonstrate the expression of BCRP in the vasculature of a significant portion of all three tumor types included in the assay. It is possible that this endothelial expression of BCRP is a specific characteristic of these tumors but it may very well also be is a remnant part of the blood-nerve-barrier of the nerves these tumors originate from. Nonetheless these results suggest that BCRP expression may reduce drug exposure to the underlying tissue in these tumors. This may be part of the reason why drug therapy of peripheral nerve sheath tumors, including vestibular schwannomas, often has variable and disappointing effects. If this hypothesis is correct, inhibiting BCRP expression in these tumors could subsequently lead to enhanced susceptibility to drug therapy.

Chapter seven forms the sequel to the analysis of the tumor associated macrophages described in chapter five. This study comprises the same patient cohort and focusses on two cytokines, M-CSF and IL-34. Both these proteins are known to have regulatory functions with regard to macrophage activation. M-CSF stands for macrophage colony stimulating factor, a cytokine that is capable of polarizing macrophages towards a M2 like phenotype. The exact tumor biological characteristics of this protein have not been fully understood yet, but there are several tumor models in which it seems play a role in macrophage associated tumor progression[11,12]. IL-34 is a cytokine that displays common features with M-CSF[13,14]. Relatively recent studies have indicated that IL-34 appears to be related to tumor progression in a manner that resembles M-CSF[15]. Our study demonstrates the expression of these two cytokines in vestibular schwannomas. In accordance with its proposed function we find that M-CSF expression is related to clinical tumor progression and the expression of M2 type macrophages. We were unable to demonstrate similar significant findlings for IL-34, but the fact that this protein has a relatively high expression in all the VS we investigated does suggest it plays a role in VS biology. These observations are in line with the hypothesis that the inflammatory microenvironment is an important factor in the progression of vestibular schwannomas. This makes M-CSF, and maybe IL-34 as well, a potential target for therapy.

Conclusion

The overall aim of this thesis is to shed more light on the biological background of the clinical progression of sporadic vestibular schwannomas. In case of vestibular schwannomas tumor progression is measured by the increase in tumor size. The obvious factors that determine vestibular schwannoma size seem to be cell growth and cystic formation.
The results of our studies indicate that, in different ways, intratumoral inflammation seems to be important in the clinical progression of these tumors. By stimulating angiogenesis and through inhibition of antitumor immune responses tumor associated macrophages may allow some tumors to progress faster and reach a larger volume. Next to permitting ongoing tumor cell proliferation it might be possible that in some tumors the actual bulk of the inflammatory infiltrate also contributes to the expansion of vestibular schwannomas.
M-CSF and IL-34 may play a regulatory role when it comes to macrophage activity within vestibular schwannomas, thereby potentially making them targets for therapy. These outcomes must be interpreted with caution. It is important to note that the results of the comparisons we made are observations of association. There is always the possibility that these findings are epiphenomena of a larger biological growth process and therefore not directly related to one another. Before treating VS patients with drugs capable of modulating the intratumoral microenvironment our findings need to be replicated, and in vitro or animal schwannoma models should be performed. Interesting examples of such drugs are PLX3397 (Pexidartinib) and RG7155 (Emactuzumab)[16,17]. Both are relatively new inhibitors capable of blocking macrophage activity and, considering our hypothesis, potentially form effective agents in vestibular schwannoma therapy.

In the search for new drugs capable of targeting tumor biological factors involved in the progression of vestibular schwannomas it is important to realize that our findings also indicate that these tumors may be protected by barrier proteins such as BCRP. Future research should reckon with this additional obstacle in order to optimize the efficacy of novel treatment strategies.

Next to looking for new pharmacologic ways to treat vestibular schwannomas there are other clinical questions and dilemmas that need to be explored. An important issue that needs further research is the potential superiority of proton radiotherapy over the conventional radiotherapy. Current literature on this topic is not decisive and clinical trials are needed to answer this question. The ongoing development of new therapeutic modalities also has its impact on shared decision making when it comes to choosing the correct form of therapy. For this reason continuous research in terms of quality of live is of great importance in order to tailor future treatment to the individual needs of the patient.

References

1. Paldor I, Chen AS, Kaye AH. Growth rate of vestibular schwannoma. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia 2016; 32:1-8.
2. Morrison H, Sperka T, Manent J, Giovannini M, Ponta H, Herrlich P. Merlin/neurofibromatosis type 2 suppresses growth by inhibiting the activation of Ras and Rac. Cancer Res 2007; 67:520-527.
3. Aarhus M, Bruland O, Saetran HA, Mork SJ, Lund-Johansen M, Knappskog PM. Global gene expression profiling and tissue microarray reveal novel candidate genes and down-regulation of the tumor suppressor gene CAV1 in sporadic vestibular schwannomas. Neurosurgery 2010; 67:998-1019.
4. Serrano C, Simonetti S, Hernandez Jet al. BRAF V600E mutations in benign and malignant peripheral nerve sheath tumors. JClinOncol 2010; 28.
5. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008; 454:436-444.
6. Solinas G, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. JLeukocBiol 2009; 86:1065-1073.
7. Allen M, Louise JJ. Jekyll and Hyde: the role of the microenvironment on the progression of cancer. JPathol 2011; 223:162-176.
8. Hartz AM, Bauer B. ABC transporters in the CNS - an inventory. Curr Pharm Biotechnol 2011; 12:656-673.
9. Brandt C, Bethmann K, Gastens AM, Loscher W. The multidrug transporter hypothesis of drug resistance in epilepsy: Proof-of-principle in a rat model of temporal lobe epilepsy. Neurobiol Dis 2006; 24:202-211.
10. Doyle LA, Yang W, Abruzzo LVet al. A multidrug resistance transporter from human MCF-7 breast cancer cells. ProcNatlAcadSciUSA 1998; 95:15665-15670.
11. Ding J, Guo C, Hu Pet al. CSF1 is involved in breast cancer progression through inducing monocyte differentiation and homing. International journal of oncology 2016; 49:2064-2074.
12. Yang L, Wu Q, Xu Let al. Increased expression of colony stimulating factor-1 is a predictor of poor prognosis in patients with clear-cell renal cell carcinoma. BMC cancer 2015; 15:67.
13. Lin H, Lee E, Hestir Ket al. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. Science (New York, NY) 2008; 320:807-811.
14. Foucher ED, Blanchard S, Preisser Let al. IL-34 induces the differentiation of human monocytes into immunosuppressive macrophages. antagonistic effects of GM-CSF and IFNgamma. PloS one 2013; 8:e56045.
15. Segaliny AI, Mohamadi A, Dizier Bet al. Interleukin-34 promotes tumor progression and metastatic process in osteosarcoma through induction of angiogenesis and macrophage recruitment. International journal of cancer 2015; 137:73-85.
16. Cannarile MA, Weisser M, Jacob W, Jegg AM, Ries CH, Ruttinger D. Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy. Journal for immunotherapy of cancer 2017; 5:53.
17. Dammeijer F, Lievense LA, Kaijen-Lambers MEet al. Depletion of Tumor-Associated Macrophages with a CSF-1R Kinase Inhibitor Enhances Antitumor Immunity and Survival Induced by DC Immunotherapy. Cancer immunology research 2017; 5:535-546.

APPENDICES

Samenvatting en conclusie
Acknowledgements
Curriculum Vitae