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REVIEW ARTICLE

New directions in molecular diagnostics and therapy of vestibular schwannomas

Nowe kierunki w diagnostyce molekularnej i terapii osłoniaków nerwu przedsionkowego (*Vestibular Schwannoma*)

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Article history: Received: 16.12.2020 Accepted: 27.12.2020 Published: 05.01.2021

ABSTRACT: The molecular basis for the formation and growth of vestibular schwannomas (VS) has been elucidated in the recent years. The main genetic and epigenetic aberrations, changes in gene expression and specific signaling pathways involved in pathogenesis of sporadic VS and neurofibromatosis type II (NF2) have been defined. These findings facilitated the search for prognostic markers in VS and potential targets for biological therapy. This publication summarizes the main directions of research in the field of molecular diagnostics and pharmacotherapy of VS based on biological agents.

KEYWORDS: acoustic neuroma, bevacizumab, biological treatment, microRNAs, prognostic markers, vestibular schwannoma

STRESZCZENIE: W ostatnich latach poznano molekularne podłoże powstawania i wzrostu nerwiaków osłonkowych nerwu przedsionkowego (ang. *vestibular schwannoma*; VS). Opisano: główne aberracje genetyczne i epigenetyczne, zmiany ekspresji genów oraz specyficzne szlaki sygnałowe, które biorą udział w patogenezie VS sporadycznych oraz związanych z neurofibromatozą typu II (NF2). Odkrycia te pozwoliły na poszukiwanie potencjalnych markerów prognostycznych VS oraz punktów uchwytu dla terapii biologicznych. Poniższa praca podsumowuje główne kierunki badań w zakresie diagnostyki molekularnej oraz terapii farmakologicznej VS, opartej na lekach biologicznych.

SŁOWA KLUCZOWE: bevacizumab, leczenie biologiczne, markery prognostyczne, mikroRNA, nerwiak osłonkowy, osłoniak przedsionkowy

ABBREVIATIONS

COX2 – cyclooxygenase 2
EGFR – epidermal growth factor receptor
HER2-4 – human epidermal growth factor receptor
NF2 – neurofibromatosis type II
PDGFR – platelet growth factor receptor
VEGF – vascular endothelial growth factor
VS – vestibular schwannomas

INTRODUCTION

In recent years, significant progress has been made in research on the molecular basis of development and growth of vestibular schwannomas (VS). The main genetic and epigenetic aberrations, changes in gene expression and specific signaling pathways involved in pathogenesis of sporadic VS and neurofibromatosis type II (NF2) have been elucidated. Current research focuses on the search for prognostic markers in VS and potential targets for biological therapies.

PROGNOSTIC MARKERS

A particularly important aspect of molecular research on vestibular schwannomas is searching for biological markers to distinguish slow-growing tumors from their more aggressive forms [1]. Developing a number of prognostic markers would facilitate monitoring of disease progression, decision-making regarding surgical treatment vs. observation, as well as the diagnosis of possible relapse. Such markers include growth factors, cytokines, receptors and other proteins, i.a. those participating in cell signaling pathways. Biological material where VS (vestibular schwannomas) markers are sought include tumor tissue, peripheral blood, cerebrospinal fluid and perilymph.

In recent years, similar to VS genome sequencing, attempts have also been made using mainly mass spectrometry techniques to determine the proteome, or a set of proteins contained in tumor tissues. Initial studies showed overexpression of apoptosis-related proteins, such as ANXA2, ANXA4, ANXA5 (*annexin-2,4,5*), Rho GDI protein (*GDP dissociation inhibitor*), HSP27 (*heat shock protein-27*) and YWHAZ protein (*zeta/delta protein*) in schwannoma

cells [2]. Xu et al. [3] analyzed the proteome of 12 VS samples and healthy vestibular nerve fragments using the iTRAQ (*isobaric tag for relative and absolute quantification*) method. On this basis, they identified 933 proteins, which exhibited significantly increased or reduced expression in tumor tissue relative to controls. These included proteins involved in cellular proliferation, such as LGALS1 (galectin-1), transcription factor STAT1, MMP3 (metaloproteinase-3), and GRB2 (*growth factor receptor-bound protein 2*). Another group of identified molecules consisted of proteins associated with apoptosis, including ANXA1, ANXA2, TRADD (*Tumor necrosis factor receptor type 1-associated DEATH domain protein*) and DIABLO [3]. Using the same method, samples of cerebrospinal fluid collected during surgery to remove VS of various clinical staging, were also analyzed. In the early stages of advancement (T1 and T2), increased expression of SCG1 proteins (secretogranin-1), KLF11 (*Kruppel-like factor 11*) and CA2D1 (*Voltage-dependent calcium channel subunit alpha-2/delta-1*) was noted in the cerebrospinal fluid. On the other hand, elevated concentrations of ABCA3 (*ATP-binding cassette sub-family A member 3*) and KLF11 as well as reduced levels of BASP1 (*Brain Abundant Membrane Attached Signal Protein 1*) and PRDX2 (*Peroxiredoxin-2*) were associated with larger tumor size. The vast majority of proteins whose expression in the cerebrospinal fluid was altered in VS patients, relative to control, belonged to the cellular pathways associated with activation of the immune system, the complement system in particular [4].

Perilymph is a fluid that fills the space between the inner wall of the bony labyrinth and the membranous labyrinth. It has been observed that in the event of inner ear damage, proteins involved in apoptosis or necrosis were secreted in large quantities into the perilymph [5]. Stankowic et al. [6] were the first to notice that hearing damage caused by VS is associated with high protein concentrations in perilymph. Subsequently, attempts were made to identify specific proteins in perilymph that may be linked to the severity of hearing loss in VS patients. Lassaletta et al. [7] identified more than a dozen proteins whose expression was altered in perilymph samples obtained from VS patients compared to those collected during cochlear implantation. Proteins with elevated expression in VS included i.a. CRYM (μ-crystalline) and LRP2 (second protein associated with low-density lipoproteins). Mutations in genes coding both of these proteins cause congenital deafness. Alpha-2-HS-glycoprotein and HSP (heat shock protein) are the examples of other proteins whose presence in perilymph has been associated with hearing loss due to VS [7].

Development of the tumor is associated with immune system activation, which is reflected by changing levels of immunomodulatory cytokines in plasma. The association between high levels of TGF-beta2 (*transforming growth factor beta*), TNF-alpha (*tumor necrosis factor alpha*) and IL-6 (*interleukin-6*) in plasma and progression of glioblastoma has also been observed. Fouladseresht et al. [8] examined the concentrations of APRIL factor (*a proliferation-inducing ligand*) in plasma of patients with VS. APRIL belongs to the TNF cytokine family, its expression stimulates proliferation of cancer cells. The study confirmed increased expression of APRIL in plasma of patients with VS, but also with meningiomas and glioblastomas, which indicates a universal role of this factor in the development of brain tumors. At present, there are no known specific markers in blood plasma that would be considered specific for VS.

NEW PHARMACOLOGICAL THERAPIES, BIOLOGICAL TREATMENT – CLINICAL TRIALS

Current strategies for the diagnosis of both sporadic and bilateral VS associated with NF2 include observation, microsurgical resection and radiotherapy. Observation is conducted in case of small, asymptomatic tumors or patients who, due to other medical conditions, cannot undergo radical treatment [9]. Treatment by microsurgical resection of the tumor is characterized by very good results with regard to preservation of facial nerve function and a very low rate of failure and relapses – less than 1% in cases of sporadic schwannomas [10]. Radiotherapy is an alternative treatment method, at the moment applied in the form of stereotactic radiosurgery with the so-called „gamma knife". This method inhibits the progression and, in some cases, also reduces the volume of the tumor. It is characterized by lower rates of facial nerve disruption than surgical treatment, and the rate of failure obtaining tumor stabilization reaches 5% for sporadic tumors [11]. Patients diagnosed with VS associated with NF2 require different therapeutic strategy than patients with sporadic VS, as these tumors often present bilaterally and are characterized by high rates of recurrence. In this group of patients, in addition to surgical and radiosurgical treatment, we are looking for pharmacological therapies that would allow preservation of hearing and a satisfactory quality of life for the longest possible time. Pharmacological treatment strategy for VS may also be effective in patients with sporadic tumors who cannot undergo radical treatment for various reasons. Thanks to the growing knowledge of molecular biology of VS, it was possible to identify potential targets for therapy. Substances that are currently undergoing clinical trials mostly include agents approved for the treatment of other types of solid tumors.

BEVACIZUMAB

Bevacizumab, an anti-VEGF antibody, was the first substance to be tested as a biological therapy therapy of VS. VEGF (vascular endothelial growth factor) is a growth factor that stimulates angiogenesis and thus, promotes tumor growth. In the first published study on the use of Bevacizumab in patients with NF2 Plotkin et al. [12] demonstrated inhibition of tumor growth and a slight improvement in hearing in 9 out of 10 patients diagnosed with VS. Three years after treatment, hearing loss remained stable in 61% of patients, while tumor size stabilization persisted in 88% of patients 3 years and in 54% of patients 5 years from the beginning of treatment. The results of Plotkin's team helped establish financing of treatment with Bevacizumab for NF2 patients in the UK. The criteria for inclusion of patients in the treatment program were established as follows: diagnosis of VS, which grows at least 4 mm or 60% of its volume over a year, potential benefits outweighing the risk of therapy [13]. These recommendations led to a wider use of Bevacizumab in the treatment of VS in NF2. Lu et al. [14] conducted a meta-analysis of 8 studies that included a total of 162 patients with NF2 and VS.

Patients were given Bevacizumab at an average dose of 5–10 mg/kg every 2–6 weeks, mean duration of treatment amounting to 14 months. During this time, tumor size reduction or stabilization was achieved in 88% of patients and stabilization or improvement of hearing in 89% of patients. At the same time, 17% of patients experienced serious side effects of therapy. Bevacizumab toxicity has been widely reported in the elderly population treated for malignancies. The most common side effects of this drug include difficult to control hypertension and proteinuria. In a meta-analysis by Lu et al. [14] the incidence of these adverse reactions was reported as 33% and 43%, respectively. Studies in the pediatric population of NF2 patients demonstrate significantly reduced efficacy of Bevacizumab compared to adults. Morris [15] and Hochart [16] demonstrated in two independent studies on small groups of patients (6 and 7, respectively) that patients under 18 showed the highest rate of tumor progression during treatment with Bevacizumab. These observations require further research on a larger group of patients.

TYROSINE KINASE INHIBITORS

Antagonists of tyrosine kinase receptors from the ErbB family comprise another group of drugs clinically tested in patients with NF2. This family includes the epidermal growth factor receptor (EGFR), platelet growth factor receptor (PDGFR) and HER2-4 receptors (human epidermal growth factor receptor). Imatinib is the first tyrosine kinase inhibitor introduced to therapy, initially of chronic myeloid leukemia and subsequently of other cancers. Altuna et al. [17] demonstrated the expression of PDGFR and c-kit receptors, which constitute the targets for Imatinib, in VS cells. Moreover, they observed concentration-dependent inhibition of proliferation of Schwannoma cell line in the presence of Imatinib [17]. Lim and Souza [18] described the case of a 30-year-old NF2 patient, in whom Imatinib was used to stabilize tumor size and hearing loss over the 4 months of follow-up. Treatment was discontinued due to the development of serious adverse reactions. Lapatinib, an EGFR/HER2 receptor inhibitor, is another tyrosine kinase inhibitor tested for VS therapy in patients with NF2. In an in-vitro model, Laptinib inhibited the ERK1/2 signaling pathway, which is dependent on AKT kinase, and thus, reduced proliferation of the Schwannoma cell line. In a subsequent phase II clinical trial, Lapatinib resulted in tumor regression and improvement in hearing of at least 10 dB in tonal audiometry in 4 out of 17 VS and NF2 patients tested [19]. Another tyrosine kinase inhibitor, Erlotinib, did not show similar effect, but only stabilized the disease in 27% of patients included in the study [20]. Paldor et al. [21] compared the efficacy of Bevacizumab, Lapatinib and Nilotinib in combination with radiotherapy in an animal model of NF2. In their study, Nilotinib or Lapatinib in combination with radiotherapy showed the greatest degree of reduction of tumor mass and a delay in its growth compared to each of the study drugs used in monotherapy and to radiotherapy. A lower proportion of serious complications requiring discontinuation of treatment with tyrosine kinase inhibitors compared to anti-VEGF antibodies must also be emphasized, making it an attractive alternative to Bevacizumab, but further clinical trials on a greater number of patients and over a longer period of time are needed [22].

mTOR KINASE INHIBITORS

Another target for VS therapy seems to be the mTOR kinase pathway, which is overactivated in schwannoma cells devoid of a normal copy of the gene coding for merlin. Despite the promising results of in-vitro studies, mTOR kinase inhibitor, Everolimus, has not demonstrated a tumor growth-inhibiting or hearing-enhancing effect in VS patients during a clinical trial [23].

ACETYLSALICYLIC ACID (ASPIRIN)

Isolated reports speak of the positive effect of acetylsalicylic acid on tumor growth inhibition and stabilization in VS patients. Behling et al. [24] retrospectively analyzed 1048 samples of VS patients, including 115 from patients with NF2, for the expression of cyclooxygenase 2 (COX2), which is inhibited by aspirin. They demonstrated COX2 expression in the tissues from all tumors, and its increased expression was associated with higher expression of the MIB1 proliferation marker. However, there was no evidence of a relationship between taking acetylsalicylic acid (Aspirin) and reduced COX2 expression in tumor tissue. In retrospective clinical trials Kandathil et al. [25] demonstrated significantly slower tumor growth in VS patients who took aspirin due to other diseases. On the other hand, in a similar retrospective study on a large group of 437 VS patients Mckeith et al. [26] failed to show such a relationship, as did Marinelli et al. [27]. However, according to the 2018 guidelines by the Congress of Neurological Surgeons, administration of acetylsalicylic acid should be considered in any patient diagnosed with sporadic VS without contraindications to antiplatelet therapy [28].

SUMMARY

The search for diagnostic markers in VS is of particular importance in the context of deciding on surgical treatment or observation, as well as monitoring of possible recurrences. Markers that could distinguish between slow-growing tumors and their more aggressive forms are now sought in the cerebrospinal fluid, perilymph and blood plasma. No diagnostic algorithm involving molecular markers that would be useful in clinical practice has been developed so far. Patients diagnosed with NF2 require a different therapeutic strategy than patients with sporadic schwannomas. Adjuvant pharmacological treatment is used in addition to surgical treatment and radiotherapy, delaying tumor regrowth and progression of hearing loss.

Currently, Bevacizumab is the only officially registered biological drug for the treatment of VS in NF2. There is ongoing research on the use of tyrosine kinase inhibitors and mTOR kinase inhibitors in the adjuvant treatment of schwannomas. There are reports of the possible effect of acetylsalicylic acid on slowing down tumor growth. Further research is needed to permit better understanding of the molecular biology of VS, making it possible to develop effective molecular diagnostic methods and biological therapies for these types of tumors.

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Word count: 2114 Tables: – Figures: – References: 28

Access the article online: DOI: 10.5604/01.3001.0014.6321 Table of content: https://otorhinolaryngologypl.com/issue/13438

Competing interests: The authors declare that they have no competing interests.

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Cite this article as: Litwiniuk-Kosmala M., Makuszewska M., Bartoszewicz R., Niemczyk K.: New directions in molecular diagnostics and therapy of vestibular schwannomas; Pol Otorhino Rev 2020; 9 (4): 55-59