

Immune Pathobiology of Schwannomas: A Concise Review

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Abstract

Schwannomas are benign tumors treatable with neurosurgery or radiosurgery, yet a small subset may exhibit aggressive growth. Hence illuminating their immune features can help develop better treatments. A tumor-promoting inflammation exists in schwannomas. Transcription factor NF- κ B triggers synthesis of inflammatory cytokines and chemokines. NF- κ B is suppressed by NF2/merlin, yet it is mutated or repressed in schwannomas, and therefore MCP-1/CCL2, MIP-1 α /CCL3, CXCL16, and CXCR6/Bonzo are likely expressed in these tumors. CD68+ and CD163+ macrophages may infiltrate schwannomas and promote their growth. Anti-inflammatory salicylates inhibit schwannomas in cell culture and clinically. Schwannomas that cannot be completely removed by neurosurgery or controlled by radiosurgery may be suitable targets of pharmacologic interventions focusing on immune mechanisms.

Keywords

- schwannoma
- merlin
- immunity
- NF- κ B
- macrophage

Introduction

General Biological and Clinical Features of Schwannomas

Schwannomas (also called neuromas or neurilemmomas) are benign and usually encapsulated tumors originating from myelin-forming Schwann cells.¹ In general, schwannomas present as solitary sporadic lesions and consist of two components: a highly ordered cellular component composed of compact spindle or oval cells arranged in interlacing fascicles (Antoni A area) and a loose myxoid and less cellular component (Antoni B area).² Schwann cells in the Antoni B area have abundant lysosomes and myelin fibers with fragmented basal lamina, indicating that they may be degenerated from Antoni A areas.³ Vestibular (acoustic) schwannomas originate from the eighth cranial nerve and localize either in the internal auditory canal or in the cerebellopontine angle.³ Vestibular schwannomas are the fourth most common intracranial tumors⁴ and exert slow but progressive growth.³ Although they are benign, they can cause substantial morbidity including sensorineural hearing loss, vestibular dysfunction, and paralysis of facial nerves.⁴ Larger tumors can lead to further paralysis of other cranial nerves and compression of the brainstem.⁴

Vestibular schwannomas are managed clinically with three strategies: microsurgery, stereotactic radiation therapy, or serial radiologic monitoring.⁵ A major problem is the differential growth pattern of these tumors, whose causes are not yet clearly defined.³ One of the problems is the large variation of tumor growth that complicates designing treatment strategies.³ Surgical resection may involve full or partial tumor removal and carries risks such as sensorineural deafness, impairment of vestibular function, paralysis of facial nerves, leakage of cerebrospinal fluid, and meningitis.⁴ Incomplete resection of vestibular schwannomas to preserve cranial nerve function necessitates deciphering novel pathologic mechanisms of aggressive schwannoma growth and developing new pharmacologic treatment strategies. We review the immunologic features of schwannomas that may improve treatment strategies.

Schwannomas contain foamy macrophages and a well-formed capsule with lymphoid aggregates that help distinguish them from malignant peripheral nerve sheath tumors.⁶ As outlined later in detail, prominent expression of proinflammatory mediators occurs in schwannomas that may contribute to tumor growth. We propose that one of the cardinal reasons for

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this feature is the loss of NF2/merlin protein. Bilateral acoustic schwannomas are diagnostic for neurofibromatosis type 2 (NF-2) syndrome, a disorder caused by germline or somatic mutations of the NF2/merlin gene on chromosome 22q12.⁷ However, Western blot and immunohistochemical studies demonstrated that sporadic (noninherited and nonbilateral) schwannomas also exert universal loss of merlin/NF2 likely via signals targeting NF2/protein to intracytoplasmic degradation.⁸ NF2/merlin is an inhibitor of NF- κ B,⁹ one of the major transcription factors inducing synthesis of proinflammatory cytokines and chemokines.¹⁰ Hence loss of NF2/merlin may induce a continuous activation of inflammatory signals within the schwannomas. This may be one reason why unilateral schwannomas and schwannomas associated with NF-2 syndrome were found to contain an equal intensity of inflammation and macrophages.¹¹ We describe the NF2/merlin association with the NF- κ B pathway later in detail.

Most studies have demonstrated that a proinflammatory microenvironment exerts many tumor-promoting effects.⁵ High numbers of cells belonging to the innate arm of immunity, such as macrophages and neutrophils invading tumors, correlate with a worse patient prognosis.¹² Innate immune cells propagate tumor growth by the production of growth factors, cytokines, chemokines, and matrix metalloproteinases, causing enhanced cell survival, invasion, angiogenesis, and suppression of adaptive immunity.¹² Degenerative changes in schwannomas include cyst formation, hemorrhage, and vascular hyalinization.² Recruitment of circulating macrophages contributes to degeneration in schwannomas.² The percentage of cyst formation in vestibular schwannomas is ~ 10%.² Cystic vestibular schwannomas behave more aggressively than their solid counterparts, with preoperative facial palsy, a short clinical history, unpredictable expansion of the cystic component, or hemorrhage.¹³ Observation alone is not appropriate for these tumors, and rapid deterioration caused by cystic expansion or hemorrhage after radiosurgery does not support radiation therapy.¹³ Abundant lymphocytes, foamy macrophages, and hemosiderin-laden macrophages exist in cystic schwannomas.¹³ Intratumoral hemorrhage introduces robust inflammatory cell infiltration, specifically macrophages, and induces the release of proteinases including matrix metalloproteinase-2 that disrupts the tumor-nerve barrier and enhances adhesion to peritumoral tissues.¹³ Fluid in these tumors is caused either by hemorrhage from hypervascular sites or by a collection of liquefied necrotic material.¹³

Macrophages in Schwannomas and Their Correlation with Tumor Behavior

Tumor-associated macrophages can be roughly classified into two types.⁵ The first type is the classically activated or M1-type inflammatory macrophages.⁵ M1-type macrophages are differentiated from monocytes via signals provided by bacterial products like lipopolysaccharides or by cytokines such as interferon- γ . Such macrophages express inflammatory cytokines such as interleukin (IL)-1 and IL-6.⁵ Monocytes differentiate to the M2 macrophages via cytokines like IL-4 and IL-13 and express scavenger receptors such as CD163 and produce IL-10, IL-1 β , and vascular endothelial growth factor (VEGF).⁵ M2

macrophages attenuate antitumor immunity and induce angiogenesis.⁵ Nonetheless, it must also be acknowledged that the M1 and M2 classification of macrophages is an oversimplification.⁵ In two consecutive studies, de Vries et al determined the association of CD68+ (a lysosome-associated glycoprotein) or CD163+ macrophages with tumor growth in schwannomas. First they assessed the correlation of leukocytes/CD45 + , hemorrhage (hemosiderin presence), and CD68 positivity with schwannoma growth. The intensity of hemosiderin and the presence of CD45+ or CD68+ cells in schwannomas were heterogeneous and associated with tumor growth and size.³ They also compared CD163 expression in 10 fast-growing versus 10 slow-growing vestibular schwannomas and found that CD163 expression and microvessel density (as assessed by CD31) were significantly higher in fast-growing tumors.⁵

CD56/NCAM and CD57 Natural Killer Cell Markers in Schwannomas

The expression of the natural killer cell (NK)-associated antigens CD56/NCAM and CD57/Leu7 was analyzed in normal and tumoral neural tissues. CD56 is identical to an isoform of the neural cell adhesion molecule NCAM.¹⁴ CD57, also termed human natural killer-1 (HNK1), is a carbohydrate epitope that contains a sulfoglucuronyl residue and exists in various adhesion molecules of neural tissues.¹⁴ Among immunocytes, CD57-positive cells are either T or NK cells.¹⁴ These antigens may also mediate cell-to-cell contacts between neural cells. Among neural tumors, CD56 was highly present in 3 of 3 benign and 8 of 13 malignant schwannomas, whereas CD57 existed only moderately in one benign (of 3) and one malignant schwannoma (of 13).¹⁴ These studies indicate that cell-to-cell contacts decrease during dedifferentiation of Schwann cells into schwannomas and/or attenuation of their immune surveillance by NK cells. However, these few studied samples hinder a definite conclusion.

NF- κ B as a Major Transcription Factor of Proinflammatory Cytokines and Chemokines: Role of NF2/Merlin in Suppression of NF- κ B

NF- κ B is a transcription factor involved in cell growth, suppression of apoptosis, and the response to and induction of inflammatory stimuli.⁹ Inflammatory cytokines induce activation of NF- κ B,⁹ and vice versa NF- κ B activates transcription of inflammatory mediators¹⁰ leading to positive inflammatory feedback. NF- κ B complexes are formed by homo- or heterodimerization of a family of proteins that includes p50, p52, p65 (RelA), c-Rel, and RelB. NF- κ B exists as an inactive complex in the cytoplasm via its binding with inhibitory proteins belonging to the I κ B family.⁹ Following NF- κ B activating stimuli, such as tumor necrosis factor (TNF) α , IL-1, and various cellular stress signals, I κ B is phosphorylated, ubiquitinated, and thereafter degraded by the 26S proteasome.⁹

NF- κ B activating inflammatory cytokines induce various kinases of signal cascades.⁹ NF- κ B inducing kinase (NIK) is one of the members of such cascades that induces the I κ B kinase (IKK) complex, a multiprotein enzyme formed by IKK α , IKK β , and IKK γ /NEMO.⁹ The activated IKK phosphorylates I κ B and

induces degradation of I κ B and subsequent nuclear translocation of NF- κ B, where it induces expression of specific genes.⁹ NIK and IKK α overexpression without NF2/merlin could induce NF- κ B-dependent transcription that can be suppressed by NF2/merlin.⁹ Additionally, NF2/merlin blocks p65, NIK, and IKK α in their transcriptional activation of NF- κ B.⁹ NF2/merlin also inhibits basal NF- κ B activity in NIH3T3 and C6 cells and TNF α -induced transactivation of NF- κ B.⁹ NF- κ B is responsible for constitutive expression of IL-1 α , IL-6, IL-8/CXCL8, and granulocyte macrophage colony-stimulating factor in head and neck cancers.¹⁰

NF- κ B mediates basal transcription of TNF α , IL-1 β , and platelet activating factor induction of cyclo-oxygenase-2 (COX-2).¹⁰ NF- κ B provides IL-1 β , VEGF, lipopolysaccharide, and TNF α induction of MCP-1/CCL-2.² NF- κ B also mediates endotoxin stimulation of MIP-1 α /CCL3.¹⁰ Lastly, NF- κ B mediates macrophage production of CXCL16,^{15,16} and vice versa CXCL16 leads activation of NF- κ B.¹⁷ Hence one of the central mechanisms responsible for the inflammatory microenvironment in schwannomas may be the loss of NF2/merlin. Next we discuss three important chemokines in schwannoma biology that all are among the transcriptional targets of NF- κ B.

Chemokine Expression in Schwannomas: MCP-1/CCL2, MIP-1 α /CCL3, CXCL16 and Its Receptor CXCR6

Chemokines are a subgroup of cytokines with similar amino acid sequences that regulate chemotaxis of immunocytes and are classified into families of C-C and C-X-C.² The C-X-C chemokines induce neutrophil chemotaxis, whereas the C-C family, including macrophage inflammatory protein-1 (MIP-1 α /CCL3), generally regulates monocytes/macrophage chemotaxis.² MIP-1 α /CCL3-positive areas correspond with CD68+ cells in Antoni B areas of schwannomas; hence they may play a role in the degenerative changes in schwannomas.² Also, the macrophage chemoattractant protein-1 (MCP-1/CCL2) and the leukemia inhibitory factor (LIF) are produced by denervated Schwann cells and the schwannoma cell lines, and both are involved in the regulation of macrophage chemotaxis.¹ Additionally, an auto-crine-signaling cascade involving IL-6, LIF, and MCP-1/CCL2 augments the Schwann cell-derived chemotactic signals.¹

Chemokines and their receptors also have a major role in tumor progression and metastasis. The chemokine CXCL16 is the ligand of CXC-chemokine receptor CXCR6/Bonzo.¹ CXCL16 exists on macrophages and dendritic cells, whereas CXCR6 exists on activated T cells, NK cells, and plasma cells.¹ CXCL16 and its receptor CXCR6/Bonzo is overexpressed in schwannoma samples as compared with normal neural tissues.¹ CXCL16 and CXCR6 mainly localize on S-100-positive schwannoma cells, and CXCL16 augments proliferation and migration of schwannoma cells.¹

Salicylate Analogs, Inhibitors of NF- κ B and Cyclooxygenases, Reduce Schwannoma Growth in Clinical and Cell Culture Studies

Salicylates including acetylsalicylic acid (universally known as aspirin) are strong suppressors of NF- κ B, and they are proposed to be used as chemotherapy sensitizing agents in cancer treatment.¹⁸ The major anti-inflammatory mechanism

of salicylates is based on inhibition of COX enzymes and subsequently the production of prostaglandins (PTGs).⁴

In 2014, by monitoring 347 vestibular schwannoma patients with serial magnetic resonance imaging, researchers found that aspirin slows schwannoma growth in patients. They demonstrated a prominent inverse association between aspirin consumption and growth of vestibular schwannomas that was not confounded by age or sex.¹⁹ Furthermore, a bioinformatics-based network investigation determined NF- κ B was a key factor in schwannoma tumorigenesis.¹⁹

A year later the same group studied the effects of salicylates on schwannoma growth in vitro.⁴ The efficacy of three different salicylates, aspirin, sodium salicylate, and 5-aminosalicylic acid, was tested against vestibular schwannomas because they are currently used clinically.⁴ All salicylates were effective in decreasing proliferation and the vitality of vestibular schwannoma cells in vitro, accompanied by reduced secreted PTG levels.⁴

Activation of DCAF1 and MEK-ERK Pathways in Schwannomas and Their Interaction with Inflammation and Immunity

In 2010, Li et al demonstrated that merlin inhibited mitogenic signaling at or near the plasma membrane.²⁰ They showed that the closed growth-inhibitory form of merlin accumulated in the nucleus binds to the E3 ubiquitin ligase CRL4^{DCAF1} and suppressed its activity. They found that the depletion of DCAF1 blocked the mitogenic action of merlin inactivation.²⁰ In contrast, enforced expression of a wild-type merlin-insensitive mutant of DCAF1 blocked the antimitogenic effect of merlin.²⁰ Reexpression of merlin and silencing of DCAF1 caused a similar tumor-suppressive program of gene expression.²⁰ Most importantly, depletion of DCAF1 inhibited the proliferation of schwannoma cells from NF2 patients and inhibited the oncogenic potential of merlin-deficient tumor cells.²⁰

It should be noted that a considerable link exists between DCAF1 activity and inflammation-dependent carcinogenesis.²¹ Chronic intestinal inflammation is closely associated with the development of colon cancer, and STAT3 plays a major role in linking chronic inflammation to the progression of colon cancer.²¹ It was shown that DICER1 was significantly downregulated in response to inflammatory IL-6 or lipopolysaccharide stimulation, and the ubiquitin ligase complex of DCAF1 was responsible for the proteosomal degradation of DICER1 and its subsequent downregulation.²¹ Noteworthy, the most prominent antitumor actions of nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin are against colon cancer.^{22–24} Furthermore, although mutations of merlin are rare in colon cancers (~8%),²⁵ its protein expression is lowered in a higher ratio (20%) of sporadic colon cancers, and this decrease is correlated with tumor dedifferentiation.²⁶

Lastly, in 2016, it was demonstrated that MEK-ERK pathway activation occurs in 10% of schwannomas, which was subsequent to in-frame *SH3PXD2A-HTRA1* fusion.²⁷ Again, aspirin and NSAIDs block invasion of human hepatoma cells and MMP-2 secretion of lung carcinoma via the blocking of MEK-ERK signaling.^{28,29} Furthermore, NSAIDs and MEK-ERK pathway inhibitors synergize in blocking the growth of liver, colon, and

endometrium cancers.^{30–32} Overall, many interactions exist between molecular pathways of schwannoma and inflammation, and it is also plausible to perform experimental studies that combine NSAIDs with DCAF1 or MEK-ERK inhibitors to block proliferation of aggressively growing schwannoma cells.

Conclusion

Understanding the immune pathobiology of schwannomas may help develop novel and more efficient management strategies for subtypes that exert more aggressive growth and lesser responses to radiotherapy. Such approaches would encompass simple applications of aspirin derivatives or more sophisticated targeted drugs against specific cytokines and chemokines. For schwannomas that cannot be completely removed by neurosurgery or controlled by radiosurgery, pharmacologic interventions focusing on immune mechanisms seem suitable targets.

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