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Cyclin D1 Expression and Histopathologic Features in Vestibular Schwannomas

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Objective: To evaluate cyclin D1 expression in vestibular schwannoma and its relationship with histologic, clinical, and radiologic features.
Patients: Twenty-one patients with histologically confirmed vestibular schwannoma.
Intervention: Immunohistochemistry analysis was performed with anticyclin D1. Histopathologic features studied included Antoni pattern and nuclear and stromal degenerative changes. Clinical charts, audiometric data, and magnetic resonance imaging characteristics were reviewed.
Main Outcome Measures: Cyclin D1 expression and its association with histologic, clinical, and radiologic findings.
Results: Cyclin D1 expression was found in 52% of cases. Cyclin D1 expression was more frequent in right-sided tumors ($p = 0.02$) and in tumors with nuclear degenerative changes ($p < 0.0001$). Patients with negative cyclin D1 expression had longer duration of deafness ($p = 0.02$) and higher 2,000-Hz hearing thresholds ($p = 0.04$) than cyclin D1+ patients.
Conclusion: Cyclin D1 expression, present in nearly half of the cases, may play a role in the development of these tumors. Further studies are needed to fully understand the contributions of histopathologic and immunohistochemical factors to vestibular schwannoma biological activity. **Key Words:** Antoni type — Cyclin D1—Degenerative changes—Vestibular schwannoma.
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The molecular changes involved in the pathogenesis of vestibular schwannoma (VS) have been studied in past years. Most studies have focused on the molecular genetic analysis of the *NF2* gene. Inactivation of merlin, the product of the *NF2* tumor suppressor gene, is responsible for VS development both in sporadic and bilateral cases (1). Epigenetic changes have also been described in several genes, some of them with clinical implications on VS behavior (2). In addition to different mechanisms of gene inactivation, deregulated expression of growth regulatory genes may also play a role in the VS tumorigenesis (3). It has been shown that cyclin D1 is a key cell-cycle regulatory protein for the mammalian G1-S phase transition and is involved in the regulation of proliferation and differentiation. Therefore, deregulated cyclin D1 expression promotes genetic instability in vitro and tumorigenesis in vivo. The role of cyclin D1 in VS development has been poorly evaluated (4).

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The aims of the present study were to analyze the association of cyclin D1 expression with histologic findings and clinical and radiologic characteristics in patients with VS.

MATERIALS AND METHODS

The study group consisted of 21 patients who underwent surgery for removal of unilateral VS in our institution between February 2002 and January 2004. There were 14 women and 7 men, with a mean age at surgery of 48 years (range, 16–71 yr). The tumor was on the left side in 13 cases (62%). Mean pure-tone threshold for the tumor ear and the unaffected ear were 42 and 23 dB, respectively. Maximum speech discrimination score ranged from 0 to 100%, with a mean of 72%. Tumor size ranged from 15 to 55 mm (mean, 29 mm). Mean tumor volume at presentation was 935 mm^3, ranging from 50 to 5363 mm^3.

The percentage of the different tissue type (A, B, mixed) in each tumor sample was analyzed. The results were grouped into 3 types: type A, greater than 60% of the tumor composed of type A tissue; type B, greater than 60% of the tumor made of type B tissue; mixed type, type A and type B tissues in equal quantity or greater than 60% of the tumor composed of type A and type B tissue. The presence of both nuclear and stromal pathologic degenerative changes was analyzed. Nuclear

changes consisted of hyperchromatic, voluminous, irregular Schwann cell nuclei. Stromal changes included hyalinized vessels, xantomathous cells, and interstitial fibrosis. Degenerative changes were graded as follows: 0, absent; ±, mild; +, moderate; and ++, severe.

The paraffin sections were dewaxed in xylene and acetone and rehydrated. Deparaffined tissue sections were incubated overnight at 4°C with the anticyclin D1 (P2D11F11) monoclonal antibody (Novocastra, Newcastle, UK) at a 1:50 dilution. Optimum primary antibody dilutions were predetermined using known positive control tissues. Multiple known positive control sections were included in each run. The tissues were incubated in biotin-labeled goat antimouse serum (1:200) for 30 minutes, rinsed with phosphate-buffered saline, and incubated with avidin-biotin-peroxidase complex for 1 hour. The signal was detected using 3,3-diaminobenzidine as the chromogen. Cyclin D1 immunoreactivity was usually nuclear, with only occasional and faint cytoplasmic immunostaining. Immunoreactivity was semiquantitatively evaluated by scoring both the staining intensity and the percentage of reacting cells. The percentage of reacting cells was evaluated by counting the stained cells in 10 high-power fields (HPF ×400). Intensity was scored 0, absent; ±, mild (<50 cells per 10 HPF); +, moderate (50–100 cells per 10 HPF); and ++, strong (>100 cells per 10 HPF).

Statistical analyses for differences between groups were performed using the Fisher's exact test and the nonparametric Mann-Whitney *U* test. Differences were considered significant at a level of $p < 0.05$.

RESULTS

Microscopic analysis showed 15 Antoni type A, 3 Antoni type B, and 3 Antoni mixed-type tumors. Nuclear and stromal degenerative changes were found in 10 (48%) and 5 (24%) tumors, respectively, with both features being present in 12 (57%) cases. No association between Antoni type and the presence of degenerative changes was found. Predominant Antoni type B tissue was more frequent in larger tumors and patients with worse preoperative hearing. In a similar way, tumors with nuclear degenerative changes corresponded to patients with longer duration of hearing loss and higher hearing thresholds for certain frequencies.

TABLE 1. *Cyclin D1 expression and clinicoradiologic findings*

	Cyclin D1−	Cyclin D1+
Age (yr)	47	50
Male (n = 7)	5 (71%)	2 (29%)
Female (n = 14)	5 (36%)	9 (64%)
Right (n = 8)[a]	1 (12%)	7 (88%)
Left (n = 13)	9 (69%)	4 (31%)
Duration of hearing loss (mo)[b]	51	10
PTA threshold (dB)	47	39
SDS (%)	62	80
250-Hz hearing threshold (dB)	39	38
500-Hz hearing threshold (dB)	37	35
1,000-Hz hearing threshold (dB)	44	39
2,000-Hz hearing threshold (dB)[c]	60	42
4,000-Hz hearing threshold (dB)	69	57
8,000-Hz hearing threshold (dB)	76	59
Tumor size (mm)	29	29
Tumor volume (mm^3)	1,194	782

[a]Cyclin D1 expression was more frequent in right tumors ($p = 0.02$).
[b]Duration of hearing loss was longer in patients with cyclin D1− tumors ($p = 0.02$).
[c]2,000-Hz threshold was higher in patients with cyclin D1− tumors ($p = 0.04$).
SDS indicates speech discrimination score.

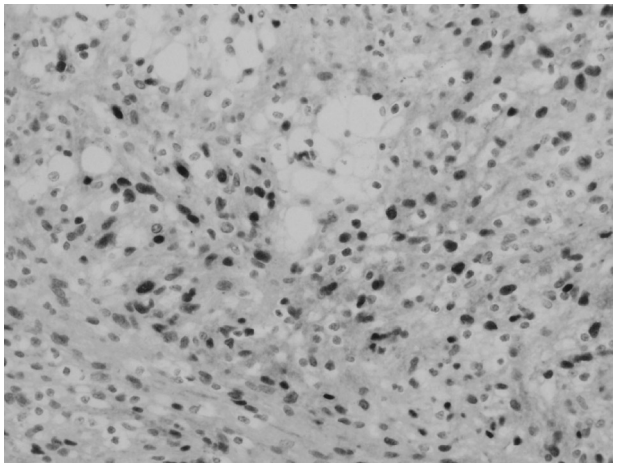

FIG. 1. Immunohistochemical localization of cyclin D1 in VS. Nuclear staining is strong in nuclei with degenerative changes. VS indicates vestibular schwannoma.

Of 21 cases (52%), 11 showed positive staining for cyclin D1. Table 1 shows the relations between cyclin D1 expression and several clinical and radiologic findings. Cyclin D1 expression was more frequent in right-sided tumors (88%) than in those tumors originating in the left side (31%) ($p = 0.02$). No difference was found between cyclin D1 expression and age, sex, main clinical complaint, preoperative facial function, and tumor size. Patients with negative cyclin D1 expression had longer duration of deafness ($p = 0.02$) and higher 2,000-Hz hearing thresholds ($p = 0.04$) than cyclin D1+ patients. Cyclin D1 expression occurred mostly in tumors with nuclear degenerative changes ($p < 0.0001$) (Fig. 1), whereas no association was found between cyclin D1 expression and Antoni type or stromal degenerative changes.

DISCUSSION

The distinction between Antoni types A and B has been associated to VS behavior. In our study, patients with predominant type B tissue had larger tumors and worse preoperative hearing in certain frequencies than those with type A or mixed type. The higher metabolic activity of type B tissue has been suggested to explain these differences in clinical behavior. However, the multifactorial pathogenesis of hearing loss, vestibular disorders, and facial paresis in patients with VS questions whether Antoni A and B patterns certainly constitute areas of functional difference. Degenerative changes are well known in VS and include both nuclear and

stromal features. The clinical significance of these changes is poorly understood. It has been postulated that the degree of degenerative changes may be correlated with tumor size (5). In our study, no association between degenerative changes and tumor size was found. However, the presence of nuclear degenerative changes was slightly associated with hearing loss.

Recent studies have established cyclin D1 as a protooncogene, revealing that its amplification and overexpression may contribute to uncontrolled cell growth in many human tumors, including mantle cell lymphoma, breast cancer, head and neck squamous cell carcinoma, and esophageal cancer. Cyclin D1 elicits its function early in G1 phase, binding to and activating the cyclin-dependent kinases CDK4 or CDK6. Active CDK 4/6-cyclin D1 complexes phosphorylate the retinoblastoma target protein Rb. Upon Rb phosphorylation, the E2F transcription factors activate the expression of S-phase genes and thereby induce cell cycle progression. However, emerging data from a number of model systems revealed that cyclin D1 also holds multiple, kinase-independent cellular functions (6). The role of the cyclin D family of proteins in VS has been recently studied. In a model of Wallerian nerve degeneration, mitotic response of Schwann cells was completely inhibited in cyclin D1 knockout mice, suggesting that cyclin D1 has a specific function in Schwann cell division (7). Seol et al. (8) suggested that the loss of p27, a regulator of the cyclin D-CDK complexes during G1 to S-phase transition, may be a predictor of VS aggressiveness. In a recent study, Neff et al. (4) found expression of cyclin D3 in 7 of 15 VS, whereas no cyclin D1 expression was found in any of these cases. No significant difference in patient demographics or tumor characteristics for the cyclin D3+ and the cyclin D3− was found.

In our study, positive expression of cyclin D1 was found in 52% of VSs. Some correlations between cyclin D1 expression and certain clinical and histologic factors were found. Tumors with positive cyclin D1 expression were more frequent in the right side. The current literature concerning VS is largely silent on the matter of tumor laterality. It is usually assumed that tumors are equally likely to occur on the left and the right side. Vestibular schwannoma laterality is receiving increasing attention in the context of concerns regarding possible association between VS and mobile phone use, with inconsistent results (9). Radiofrequency radiation increases the expression of mitogenic signal transduction genes in human skin fibroblasts (10). Although an association between VS and certain protooncogen markers such as cyclin D1 can be related to mobile phone exposure, no evidence between the use of cellular phones and the risk of developing VS has been fully established (9).

A slight tendency was found between worse preoperative hearing and negative cyclin D1 expression, and tumors with nuclear degenerative changes were highly associated with negative cyclin D1 expression. These results suggest that cyclin D1 expression plays a role in VS behavior. It seems to be a slight association between long-standing tumors (longer duration of hearing loss and higher thresholds in certain frequencies) and degenerative changes, as well as between nuclear degenerative changes and cyclin D1 expression. To our knowledge, this study is the first to assess the clinical and histologic implications of cyclin D1 expression in VS. Because of the small size of our series, further studies in large prospective cohorts are now required to confirm these findings and fully establish any further clinical significance of these events.

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