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Cyclin D1 Expression and Facial Function Outcome After Vestibular Schwannoma Surgery

*Luis Lassaletta, *Laura Del Rio, †Miguel Torres-Martin, †Juan A. Rey, ‡Mercedes Patrón, §Rosario Madero, ||Jose Maria Roda, and *Javier Gavilan

*Departments of *Otolaryngology, †Unidad de Investigación, Laboratorio Oncogenetica Molecular, ‡Pathology, §Statistics, and ||Neurosurgery, Hospital La Paz/Autónoma University School of Medicine, IdiPAZ, Madrid, Spain*

Hypothesis: The proto-oncogen cyclin D1 has been implicated in the development and behavior of vestibular schwannoma. This study evaluates the association between cyclin D1 expression and other known prognostic factors in facial function outcome 1 year after vestibular schwannoma surgery.
Methods: Sixty-four patients undergoing surgery for vestibular schwannoma were studied. Immunohistochemistry analysis was performed with anticyclin D1 in all cases. Cyclin D1 expression, as well as other demographic, clinical, radiologic, and intraoperative data, was correlated with 1-year postoperative facial function.
Results: Good 1-year facial function (Grades 1–2) was achieved in 73% of cases. Cyclin D1 expression was found in 67% of the tumors. Positive cyclin D1 staining was more frequent in patients with Grades 1 to 2 (75%) than in those with Grades 3 to 6 (25%). Other significant variables were tumor volume and facial nerve stimulation after tumor resection. The area under the receiver operating characteristics curve increased when adding cyclin D1 expression to the multivariate model.
Conclusion: Cyclin D1 expression is associated to facial outcome after vestibular schwannoma surgery. The prognostic value of cyclin D1 expression is independent of tumor size and facial nerve stimulation at the end of surgery. **Key Words:** Acoustic neuroma—Cyclin D—Facial nerve—Facial paralysis—Tumor size—Vestibular schwannoma.
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With the advent of sophisticated microsurgical and anesthetic techniques, morbidity and mortality have dramatically decreased in vestibular schwannoma (VS) surgery. Advances in imaging techniques, which allow for earlier diagnosis, and routine use of intraoperative cranial nerve monitoring, have led all surgeons to attempt for conservation of facial function. Maintenance of facial nerve integrity is usually achieved in more than 95% of cases. However, preservation of facial function is usually lower than anatomic preservation. Achievement of an excellent result (House-Brackmann grade 1 or 2) depends on hospital volume and varies from 43% to 98% (1).

In addition to a meticulous surgical technique, a variety of factors may affect facial nerve outcome (2). Preoperative factors include age of the patient, tumor size, previous treatment, preoperative facial function, and magnetic resonance imaging (MRI) appearance. Stimulation of the facial nerve after tumor removal, surgical approach, adherence of the tumor to the facial nerve, and displacement pattern of the facial nerve have been suggested as prognostic intraoperative factors (3). Antoni type and tumor edema have been described as postoperative prognostic factors (4). However, there is still controversy about the importance of all these factors in terms of prediction of postoperative facial function for a particular patient.

Recent studies suggest an important role for the Rb-CDK pathway in VS development and behavior (5–7). Immunohistochemical cyclin D1 expression has been recently described in 52% of 22 patients with VS. Cyclin D1 expression was more frequent in tumors with nuclear degenerative changes, as well as in patients with longer duration of deafness and higher (2,000 Hz) hearing thresholds (8).

In this study, we attempt to analyze the association between cyclin D1 expression and 1-year postoperative facial function in patients undergoing VS surgery. The role of other preoperative, intraoperative, and postoperative factors also is analyzed.

Address correspondence and reprint requests to Luis Lassaletta, M.D., Servicio de ORL. Hospital La Paz. P. de la Castellana, 261. 28046 Madrid, Spain; E-mail: luikilassa@yahoo.com

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MATERIALS AND METHODS

Patient Selection

Among 106 patients who underwent VS surgery between January 2001 and January 2006, 64 patients were included in this study. Exclusion criteria included lost to follow-up (n = 10), history of irradiation (n = 4), previous VS surgery (n = 5), facial nerve reconstruction (n = 3), patients operated by occasional surgeon (n = 5), and no tissue available for immunohistochemistry (n = 15).

Patients

The population included 37 women and 27 men with a mean age at surgery of 49 years (range, 16–78 yr). The tumor was on the left side in 32 cases (50%). The main complaints at the time of diagnosis were hearing loss (52%), tinnitus (22%), dizziness (12%), vertigo (6%), and other symptoms (8%). The preoperative facial function was Grade 1 in 91% of the patients and Grade 2 in 9%. Mean pure-tone threshold for the tumor ear and the unaffected ear was 51 and 24 dB, respectively. Maximum speech discrimination score ranged from 0% to 100%, with a mean of 58%. Tumor size measured as the largest diameter in the axial plane of MRI ranged from 5 to 55 mm (mean, 23 mm). Size was evaluated as Stage 1 (intracanalicular) in 6 cases (9%), Stage 2 (15 mm in its greatest diameter in the cerebellopontine angle [CPA]) in 22 cases (34%), Stage 3 (16–30 mm in the CPA) in 28 cases (44%), and Stage 4 (>30 mm in the CPA) in 8 cases (12%). In addition, the tumor volume also was measured from MRI, using the formula for an ellipsoid, as previously described (9). Mean tumor volume at presentation was 6.39 cm^3, ranging from 0.12 to 53.63 cm^3. Tumor appearance was homogeneous (69%), heterogeneous (14%), and cystic (16%). Tumor in the CPA was centered to the internal auditory canal in 77%, posterior in 20%, and anterior in 3% of the cases.

Surgical Procedures and Outcome Measures

Surgical approach was retrosigmoid in 39 cases (61%), translabyrinthine in 23 cases (36%), and through the middle fossa in 2 cases (3%). Two neurotologist surgeons (J. G. and L. L.) and a neurosurgeon (J. M. R.) were involved in all the surgical procedures. The facial nerve was monitored intraoperatively, using the Nerve Integrity Monitor II facial nerve monitor (Medtronics-Xomed, Jacksonville, FL, USA). The facial nerve was electrically stimulated by a monopolar probe near the brainstem after tumor removal. The intensity threshold that elicited a response greater than 100 μV was classified into 4 categories (≤0.05, 0.06–0.1, and ≥0.2 mA and no response). All patients underwent gross resection of their tumors. Microscopic disease was left on the facial nerve in 10% of cases. Postoperative facial function by means of the House-Brackmann grading system (10) was recorded 1 day, 1 week, and 1 year postoperatively.

Immunohistochemistry

Tumor tissues obtained at surgery were fixed in 10% formalin and embedded in paraffin. Staining with hematoxylin-eosin was performed for routine microscopic diagnosis. 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. Only cells with brown-colored nuclear staining were considered as positive. The vestibular nerves of the 3 patients undergoing vestibular neurectomy for Ménière's disease were used as controls.

Statistical Analyses

Categorical variables were described by frequency (percentage) and continuous variables as mean (standard deviation). Groups of patients were compared using the *t* test for continuous symmetrically distributed variables, the Mann-Whitney test for continuous asymmetrically distributed variables, and χ^2 and Fisher exact tests for categorical values. Odds ratio (OR) and difference in means were used as measures of effect. To analyze the effect of cyclin D1 on 1-year facial outcome, a binary logistic regression was performed. Both a nonadjusted model and an adjusted model to control for confounding variables (tumor volume and facial nerve stimulation threshold) were done. To determine the performance of cyclin D1 expression in predicting facial outcome, we estimated the area under the receiver operating characteristic (ROC) curve. A leave-one-out cross-validation (LOOCV) was used as an internal validation method. All analyses were performed using SPSS 13.0 for Windows. Differences were considered significant at a level of $p < 0.05$. La Paz University Hospital institutional review board approval was obtained for this study.

RESULTS

Facial Function and Cyclin D1 Expression

Facial function 1-day, 1-week, and 1-year postoperatively are summarized in Table 1. One-year postoperative good results (Grades 1–2) were achieved in 73% of patients. Positive staining for cyclin D1 was observed in 43 cases (67%) (Fig. 1). A significant association was found between cyclin D1 expression and 1-year facial function. Cyclin D1 expression was more frequent in patients with Grades 1 to 2 (75%) than in those with Grades 3 to 6 (25%). Negative cyclin D1 expression was associated with worse 1-year facial outcome (OR, 3.28; 95% confidence interval [CI], 1.03–10.43, $p = 0.039$).

Preoperative Factors and Their Relationship to Postoperative Facial Function

The percentage of patients with 1-year facial postoperative Grades 1 to 2 was 100% in Stage 1, 82% in Stage 2, 71% in Stage 3, and 37% in Stage 4 (linear trend, $p = 0.007$). Mean tumor diameter of patients with postoperative Grades 1 to 2 and 3 to 6 were 20.7 and 29.1 mm, respectively ($p = 0.009$). Mean tumor volume of patients with postoperative Grades 1 to 2 and 3 to 6 were 3.79 and

TABLE 1. *Postoperative 1-day, 1-week, and 1-year facial function*

	Grades 1–2	Grades 3–4	Grades 5–6
1 d postoperative	40 (62%)	11 (17%)	13 (20%)
1 wk postoperative	36 (56%)	11 (17%)	17 (26%)
1 yr postoperative	47 (73%)	13 (20%)	4 (6%)

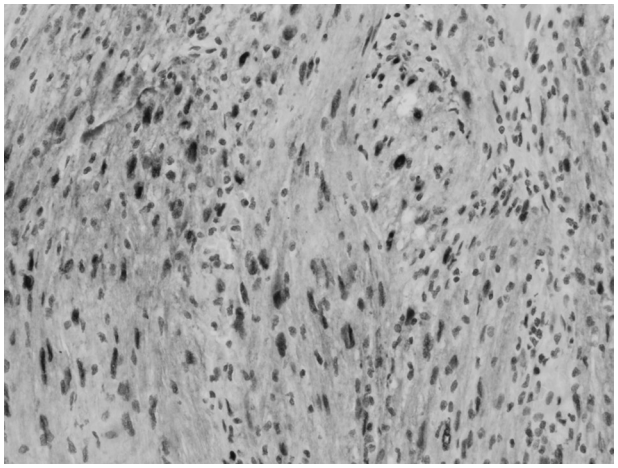

FIG. 1. Strong positive cyclin D1 staining in a tissue section of a vestibular schwannoma.

11.98 cm^3, respectively ($p = 0.003$). No association was found between preoperative hearing and 1-year postoperative facial function. Interestingly, an association was found between preoperative hearing and postoperative immediate facial function. Patients with lower 250-Hz and 500-Hz preoperative thresholds had better 1-day postoperative facial function ($p = 0.015$ and $p = 0.05$, respectively). There was no association between postoperative facial function and age, sex, tumor side, preoperative symptoms, preoperative facial function, tumor appearance, and tumor position (data not shown).

Intraoperative Factors and Their Relationship to Postoperative Facial Function

No association was found between surgical approach and postoperative facial function. Table 2 shows facial outcome as a function of intraoperative facial nerve stimulation. Patients with a stimulation threshold of 0.05 or lower had a higher proportion of good facial function at 1 year postoperatively in comparison with those with a higher threshold ($p < 0.001$).

Postoperative Factors and Their Relationship to Postoperative Facial Function

One-day and 1-week postoperative facial function were associated with 1-year postoperative function ($p < 0.001$ in both cases). Ninety-two percent of patients with 1-day postoperative Grades 1 to 2 and 94% of patients with 1-week postoperative Grades 1 to 2 achieved Grades 1 to 2 one year postoperatively.

TABLE 2. *Postoperative 1-year facial function and facial nerve stimulation after tumor removal*

	≤0.05 mA	0.06–0.1 mA	≥0.2 mA	No response
Grades 1–2	44 (94%)	3 (6%)		
Grades 3–6	9 (53%)	2 (12%)	3 (18%)	3 (18%)
Total	53 (83%)	5 (8%)	3 (5%)	3 (5%)

TABLE 3. *Logistic regression of study variables for worse 1-year facial outcome (adjusted model)*

		95% Confidence interval		
Variable	Odds ratio	Lower	Upper	*p*
Tumor volume (mm^3)	1.11	1.00	1.23	0.041
Stimulation threshold >0.05	14.16	2.36	84.94	0.004
Negative cyclin D1 expression	4.07	0.91	18.17	0.066

Multivariate Analysis

To analyze the independent effect of cyclin D1 expression, tumor volume, and facial stimulation for facial outcome at 1 year postoperatively, a multivariate binary logistic regression was performed. The model showed an increase in predicting worse facial outcome for negative cyclin D1 expression when adjusting by volume and facial stimulation (OR, 4.07; 95% CI, 0.91–18.17) (Table 3). The ROC curves are represented in Figure 2. The area under the curve for the multivariate model measures the capacity of discrimination between good and bad facial outcome 1 year after surgery. The area under the curve was 0.784 (95% CI, 0.641–0.92) for tumor volume and facial nerve stimulation threshold. Adding cyclin D1 expression to the predictive model, we obtained a greater area under the curve that was 0.823 (95% CI, 0.693–0.959). We validated our model using the LOOCV method, obtaining an area under the curve of 0.742 (95% CI, 0.582–0.900).

DISCUSSION

Preservation of facial function remains one of the most important considerations of patients undergoing VS surgery (11). There are several factors that may influence facial outcome. In this study, we have confirmed the

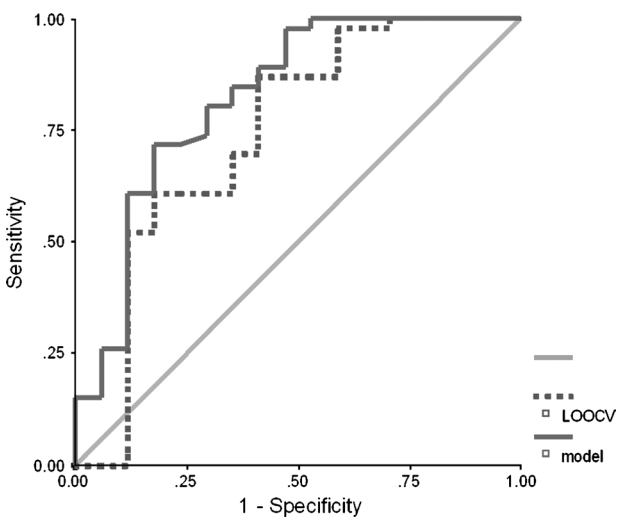

FIG. 2. ROC curves for the model (*dotted line*) and for the validation of the model using the LOOCV method (*solid line*).

prognostic usefulness of 2 well-known factors, such as tumor size and facial nerve stimulation after tumor removal (12–14).

The effect of tumor size on facial nerve function has been extensively reported. As a general rule, most surgeons agree in that the larger the tumor, the poorer the facial function outcome. Our results and previous studies suggest that tumor volume may be more useful than tumor diameter in predicting clinical findings (15,16).

Intraoperative facial monitoring has become a standard and essential tool in VS surgery. The stimulation threshold at the root entry zone of the facial nerve after tumor removal is one of the strongest prognostic factors for long-term facial outcome (17). A threshold between 0.01 and 0.04 had a positive predictive value of 94% for good facial function (Grades 1 or 2) in a study by Grayeli et al (18). In a similar way, 90% of our patients with a stimulation threshold of 0.05 or lower had facial Grades 1 to 2 one year after surgery. One of the main advantages of facial nerve monitoring is that its prognostic value is independent of other preoperative factors, that is, a low stimulation threshold at the end of the resection reflects a favorable prognosis irrespective of tumor size, tumor type, tumor position, or grade of resection.

In addition to these well-known prognostic factors, we found that positive cyclin D1 expression was associated to 1-year facial outcome, the association being independent of tumor size and facial nerve stimulation. To our knowledge, this study represents the first analysis studying the association between cyclin D1 expression and facial nerve outcome after VS surgery. The small number of patients in this series makes us cautious about the meaning of the results. Nevertheless, on multivariate analysis, the OR of positive cyclin D1 expression was slightly larger than that of univariate analysis, still independent of tumor volume and facial nerve stimulation, and approaching statistical significance.

Several studies have established cyclin D1 as a proto-oncogene, revealing that its amplification and overexpression may contribute to uncontrolled cell growth in many human tumors (19). However, the significance of immunohistochemical expression of a proto-oncogen in a benign tumor, such as VS, remains unknown. Mutation of the NF2 gene product merlin can be detected in NF-2–related and sporadic schwannomas, supporting its role as a tumor suppressor gene. Xiao el al. (20) have shown that cyclin D1 is an essential mediator of merlin's effect on cell cycle progression because merlin can repress cyclin D1 through inhibition of PAK. The authors suggest that how a tumor suppressor gene contributes to both malignant mesotheliomas and benign schwannomas may seem paradoxical and that a mechanism other than tumor invasiveness must be invoked to attribute a role for NF-2 inactivation in both malignant and benign tumors. Lü et al. (21) performed in situ hybridization immunohistochemistry and Western blot in tissue specimens from 54 VS patients. They found that merlin gradually moved from the cytoplasm to the nucleus, whereas cyclin D1 shifted from the nucleus to the cytoplasm, indicating a transfer of the cycle from G1 to S phase. They also suggest a different behavior of merlin in benign and malignant tumors.

In our study, we found a significant association between cyclin D1 expression and 1-year facial function. Patients with positive cyclin D1 expression had better facial function outcome. In a previous study, we found an association between cyclin D1 expression and cellular degenerative changes (8). As these changes may reflect long-term, that is, less aggressive lesions, this could explain why overexpression of a proto-oncogen is associated with a favorable facial outcome. Atanasoski et al. (22) studied the role of cyclin D1 as a key regulator of the cell cycle in Schwann cells. They found that cyclin D1 increased in Schwann cells after axonal damage caused by trauma, neuropathies, or tumors, such as schwannomas, suggesting that injury-induced Schwann cell proliferation is cyclin D1 dependent. Our findings suggest that the characteristics of the tumor that lead to better or worse facial outcome also may be cyclin D1 dependent. This could explain the worse facial function outcome of patients with negative cyclinD1 tumors, irrespective of well-known factors as tumor size or facial nerve stimulation threshold.

In conclusion, the results of this study suggest that positive cyclin D1 expression is associated with better long-term facial outcome after VS surgery. Its effect is independent of tumor size and facial nerve stimulation after tumor resection. 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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