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Retinoblastoma–Cyclin-Dependent Kinase Pathway Deregulation in Vestibular Schwannomas

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Objectives: **The purpose of the study was to identify genes of the retinoblastoma protein (pRb)–cyclin-dependent kinase (CDK) pathway that are deregulated in vestibular schwannomas when compared with normal vestibular nerve tissues.** ***Study Design:*** **Expression profiles in eight vestibular schwannomas (four sporadic tumors, one neurofibromatosis type 2 tumor, and three cystic tumors) and a paired normal vestibular nerve from one of the eight patients were chosen. Genes examined included the retinoblastoma susceptibility gene (Rb-1); cyclins D1, D2, A, and E; the CDK inhibitors p18, p19, and p27; CDK2 and CDK6; transcription factors E2F-4, E2F-5, and DP-1; and the neurofibromatosis type 2 gene.** ***Methods:*** **Total RNA samples were extracted from normal vestibular nerve and vestibular schwannoma tissues and used to generate radiolabeled complementary DNA (cDNA) samples. Labeled cDNA probes were then hybridized to cDNA microarray filters. The hybridization signal was captured and quantified. Differential gene expression profiles between the normal vestibular nerve and vestibular schwannoma were compared. Real-time polymerase chain reaction and immunohistochemistry were used to further confirm the cDNA microarray data.** ***Results:*** **Among genes in the pRb-CDK pathway, CDK2 was substantially underexpressed in seven of the eight vestibular schwannoma tumors examined. Quantitative RNA expression analysis using real-time polymerase chain reaction also showed consistent downregulation of CDK2 in the tumors. Anti-CDK2 antibody stained predominantly in the vestibular nerve and ganglion cells but only weakly in the vestibular schwannoma tissues.** ***Conclusions:*** **The pRb-CDK pathway was altered in all vestibular schwannoma tumors examined, with CDK2 significantly downregulated in seven of the eight tumors. Further investigation into the regulatory mechanisms governing CDK2 expression may lead to a better understanding of vestibular schwannoma tumorigenesis.** ***Key Words:*** **Vestibular schwannoma, acoustic neuroma, retinoblastoma, retinoblastoma protein–cyclin-dependent kinase pathway, DNA microarray, immunohistochemistry, real-time polymerase chain reaction.**

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INTRODUCTION

Patients with neurofibromatosis type 2 (NF2) have an inherited predisposition to the development of central and peripheral nervous system tumors. The hallmark of NF2 is the formation of bilateral vestibular schwannomas; however, other cranial nerve schwannomas, meningiomas, and ependymomas are also observed with increased frequency in these patients. Vestibular schwannomas cause significant morbidity including tinnitus, hearing loss, cranial neuropathies, and balance disturbance. Continued tumor growth can lead to brainstem compression, hydrocephalus, and death.[1]

Vestibular schwannomas are most commonly sporadic, resulting from Schwann cell transformation within the vestibular division of the eighth cranial nerve. It is thought that spontaneous mutations inactivating both alleles of the neurofibromatosis type 2 gene *(NF2)* are responsible for the development of these sporadic unilateral tumors. Patients with NF2 inherent one mutated copy of the *NF2* gene through autosomal dominant transmission. Inactivation of the second allele results in the loss of *NF2* tumor suppressor function. Furthermore, approximately 95% of patients with NF2 develop bilateral vestibular schwannomas.

The *NF2* gene has been mapped to chromosome 22q12 and encodes a cytoskeletal protein named schwannomin,[2] or merlin,[3] that appears to have a role in modulating cellular motility and proliferation. Overexpression

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of the normal but not mutant *NF2* gene inhibits actin-cytoskeleton–mediated processes including cell motility, cell spreading, and cell attachment.[4] These data suggest a tumor suppressor role for schwannomin/merlin. The control of Schwann cell proliferation is lost by the inactivation of the *NF2* gene, which suggests that schwannomin/merlin deficiency disrupts some aspect of intracellular signaling that leads to cellular proliferation.[5,6] How *NF2* functions as a tumor suppressor and its interactions with other cellular proteins in Schwann cells are poorly understood.

The mammalian cell cycle is divided into four phases: G_1, S (DNA replication), G_2, and M (mitosis).[7] It has been shown that G_1- to S-phase progression is tightly regulated by the retinoblastoma protein–cyclin-dependent kinase (pRb-CDK) pathway (Fig. 1). Hypophosphorylated pRb and its family members p107 and p130 bind to the E2F transcription factor whose binding sites are present in several genes necessary for G_1- to S-phase progression and DNA replication. Hypophosphorylated pRb/E2F complexes repress these genes through the E2F-binding sites in their promoters, thereby downregulating their expression and consequently blocking cell cycle progression at G_1.

The progression of the cell cycle is regulated by a group of protein kinases, termed cyclin-dependent kinases.[8] The CDK-associated kinase activities depend on the synthesis of and association with specific regulatory subunits known as cyclins. When quiescent cells are stimulated to enter the cell cycle, expression of both cyclin D and cyclin E are induced. The cyclin D–associated CDK4/CDK6 kinase activity reaches a maximum at mid G_1 phase, whereas the cyclin E-associated CDK2 kinase activity reaches a maximum at late G_1 to early S phase. The cyclin-CDK complexes hyperphosphorylate pRb and release the E2F transcription factor from its complexes. The E2F transcription factor is a heterodimer consisting of one of the six E2F members (E2F-1–E2F-6) and one of the two associated DP proteins (DP-1 and DP-2). Free E2F can then transcriptionally activate the genes necessary for cycle progression.

The CDK activities are also regulated by a group of CDK inhibitors.[8] There are two families of CDK inhibitors. The Cip/Kip family includes p21, p27, and p57 and influences cyclin-CDK complexes during G_1- to S-phase transition. The INK family includes p15, p16, p18, and p19 and inhibits cyclin-CDK4/CDK6 complexes.

Alterations in growth regulatory genes have been frequently observed in human tumors.[7] Several genes in the pRb-CDK pathway have been implicated in human tumorigenesis; however, alteration in these genes in vestibular schwannomas has not been examined.

The cDNA microarray technology is a tool for analyzing gene expression in a large-scale fashion.[9] The expression profiles of thousands of genes in a tumor can be compared with the normal tissue of origin. Genes that are upregulated or downregulated in several human carcinomas have been identified and evaluated for their relation to growth regulation and specific signaling pathways.[10–13] The goal of the present research is to employ cDNA microarray analysis, real-time polymerase chain reaction (PCR), and immunohistochemical methods to identify growth-regulatory genes, specifically members of the pRb-CDK pathway, which are deregulated in vestibular schwannomas.

MATERIALS AND METHODS

Tissue Procurement and RNA Isolation

The institutional review board approved the human subject protocol for the present investigation. Eight patients with vestibular schwannomas were included in the study. Four patients had sporadic unilateral schwannomas, three had sporadic cystic schwannomas, and one had a schwannoma associated with NF2. The tumors ranged in size from 1.5 to 3.0 cm in diameter. All eight patients had non-serviceable sensorineural hearing loss

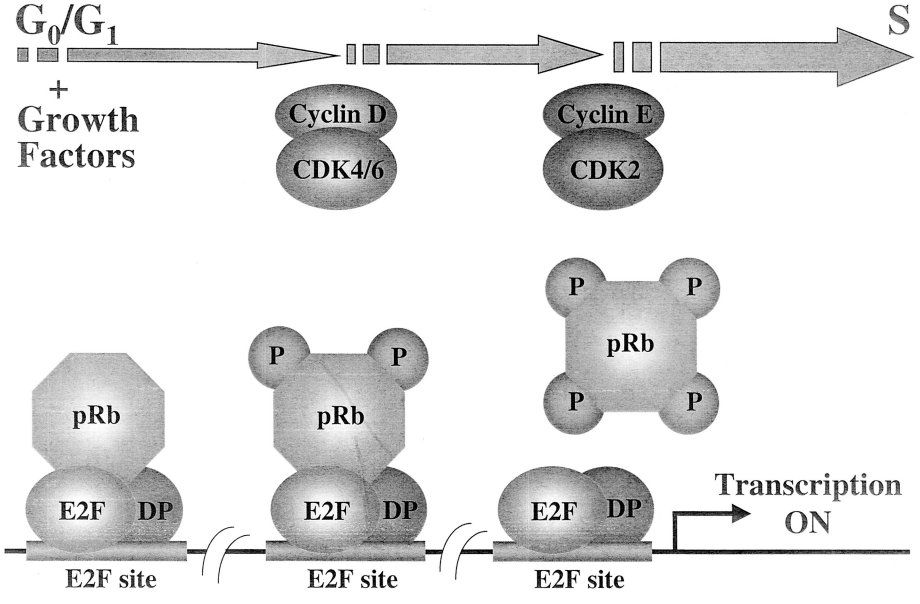

Fig. 1. Schematic diagram of the retinoblastoma protein–cyclin-dependent kinase (pRb-CDK) pathway during G_1- to S-phase progression. On addition of growth factors, quiescent cells are stimulated to enter the cell cycle by the induction of cyclin-CDK associated kinase activities that phosphorylate pRb (indicated by the addition of "P" to the protein). Hyperphosphorylation of pRb results in the release of the E2F transcription factor from its complexes. Free E2F can then transcriptionally activate the genes necessary for cell cycle progression.

with poor discrimination scores on the side of the tumor and had a translabyrinthine resection.

Tissue procurement entailed meticulous dissection with the operating microscope during surgery to separate vestibular schwannomas from the normal vestibular nerve tissue. A paired normal vestibular nerve was also harvested from the division opposite the origin of a 1.5-cm vestibular schwannoma (VS) to eliminate any tumor tissue contamination. In addition, any surrounding tissue was dissected away from the nerve. The VS samples were taken from the central portion of the tumor. Arachnoid tissues surrounding the tumor were specifically avoided and removed. Histopathological examination confirmed that each tumor sample was, indeed, a VS.

On surgical removal, all specimens were immediately frozen in liquid nitrogen to prevent degradation of tissue RNA samples. Total RNA samples were isolated from the VS and normal vestibular nerve using TRIzol reagent, according to the manufacturer's recommendations (Life Technologies). RNA samples then underwent DNAse treatment to remove any contaminating genomic DNA. Optical density readings determined the concentration of RNA samples. The quality of RNA was determined by electrophoresis on a 1% agarose gel, identifying the 18S and 28S ribosomal RNA bands.

cDNA Microarray Analysis

The cDNA Microarray Genefilters were purchased from Research Genetics. Each gene filter contains more than 5000 genes or expressed sequence tags (ESTs), and each tissue sample was hybridized with five different filters. Therefore, more than 25,000 known genes and ESTs were examined for each tissue sample. Duplicate filters were used for each VS sample and normal vestibular nerve.

The Genefilters were prehybridized with 20 mL MicroHyb (Research Genetics) containing 20 μg human cot-1 DNA and 10 μg poly(dA) in a roller tube at 42°C. RNA (1–10 μg) was reverse transcribed using Superscript II reverse transcriptase (Life Technologies) in the presence of [P^{33}]deoxycytidine triphosphate to produce the labeled cDNA probe. After purification through a BioSpin 6 column (BioRad), the cDNA probe was hybridized with the Genefilters at 42°C overnight. Then, the filters were washed twice with 2X SSC and 1% SDS (sodium dodecyl sulfate) at 50°C for 20 minutes and once with 0.5X SSC and 1% SDS at 55°C for 15 minutes. The Genefilters were placed on a Whatman 3MM paper moistened with 0.1% SSC and wrapped with plastic wrap. The wrapped filters were exposed to a phosphoimager screen, and the hybridization signals were captured using a Storm 860 phosphoimager (Molecular Dynamics) at 50-μm resolution. The Pathways software (Research Genetics) was used to analyze the gene expression data.

Real-Time Polymerase Chain Reaction Technique

The primers and probes for CDK2 were designed using the Primer Express software (Applied Biosystems) and are shown in Table I. The RNA samples from the normal vestibular nerve and VS tumors were reverse transcribed with Superscript II reverse transcriptase to produce cDNA. Standard PCR was used to verify that the designed primer pair amplified the correct-sized product with each VS cDNA sample. The designed primer/probe pair was then used in real-time PCR analysis of the cDNA samples using an ABI PRISM 7700 Sequence Detection System under the same PCR cycle conditions. Approximately 125 ng of each cDNA sample was added to Taqman Universal Master Mix (Applied Biosystems) containing 9 μmol/L of each primer and 2 μmol/L probe. Amplification of human β-actin cDNA was included as the endogenous control to normalize any difference in the amount of cDNA sample used and any inhibition of the reaction caused by the sample.

TABLE I.
Primers and Probes for Real-time PCR Analysis.*

	CDK2
Forward primer	CAAATGCTGCACTACGACCCTA
Probe	FAM-AGCGACGAGCATGTCCGCACA
Reverse primer	GAAAGGGTGAGCCAGGGC

*FAM is the reporter dye attached to the probe for real-time PCR.

The ABI PRISM Sequence Detection System software was used to analyze the data generated with real-time PCR. Quantitative expression data of the specific target were obtained for each cDNA sample. The comparative threshold of cycle (C_T) method was then used to determine any difference in target expression between the normal vestibular nerve and VS samples using β-actin as the endogenous control.

Immunohistochemical Analysis

Immunohistochemical analysis was employed to examine the expression of the CDK2 protein within a surgical specimen containing both schwannoma and vestibular nerve tissues. Paraffin-embedded sections were mounted on positively charged slides. The slides were heated for 1 hour at 60°C, cooled, and treated with xylene and graded ethanol solutions for deparaffinization and rehydration. To block endogenous peroxidase activities, the samples were treated with 3% hydrogen peroxide solution in methanol for 5 minutes. The slides were then placed in a citric-acid target retrieval solution (Dako) at 94°C for 30 minutes for antigen retrieval.

A labeled streptavidin-biotin complex was used to conjugate and stain the CDK2 protein using a Dako autostainer. Briefly, tissue sections were incubated with anti-CDK2 antibody (Santa Cruz Biotechnology) at 1:300 dilution for 60 minutes. After extensive washing, the slides were sequentially treated with a biotinylated-linking antibody for 20 minutes, conjugated streptavidin for 20 minutes, and substrate chromogen for 5 minutes. Hematoxylin counterstain was then applied. The slides were dehydrated with graded ethanol solutions and dried overnight. Light microscopy was used to visualize the samples. Tissue expressing the CDK2 protein stained brown, and the hematoxylin counterstain appeared blue.

RESULTS

Patient Description

Eight patients with vestibular schwannomas were included in the present study. There were six men and two women, and their ages ranged from 19 to 67 years. Four patients had spontaneous unilateral schwannomas and three had cystic tumors noted both on preoperative imaging and during surgery. One patient had NF2 and bilateral schwannomas. In addition, the uninvolved superior vestibular nerve was harvested from the patient with a small VS of the inferior vestibular nerve.

cDNA Microarray Analysis

The gene expression profiles from the paired normal vestibular nerve and the VS tumor from the same patient were first compared. After normalization with the internal controls, genes that showed a threefold or greater difference in the level of RNA expression be-

TABLE II.
The pRb-CDK Pathway Gene Panel.

Gene	Protein Product
Rb-1	pRb Protein
CDK2	Cyclin-dependant kinase 2
CDK6	Cyclin-dependent kinase 6
cyclin D1	Forms CDK4/6 complex
cyclin D2	Forms CDK4/6 complex
cyclin E	Forms CDK2 complex
cyclin A	Forms CDK2 complex
p18	CDK inhibitor (CDK4/6)
p19	CDK inhibitor (CDK4/6)
p27	CDK inhibitor (CDK2 and CDK4/6)
E2F-4	E2F-4 transcription factor
E2F-5	E2F-5 transcription factor
DP-1	Forms a heterodimer with E2F
NF2	Schwannomin/merlin

tween the paired normal vestibular nerve and schwannoma were considered for further evaluation. Approximately 0.5% of the 25,920 genes or ESTs surveyed displayed a threefold or greater difference in expression levels between the normal vestibular nerve and tumor. Several were noted to be members of the pRb-CDK pathway. A detailed search for the pRb-CDK pathway genes among the 25,920 genes or ESTs that were examined identified 14 members of this pathway (Table II). These included the retinoblastoma susceptibility gene (Rb-1); cyclins D1, D2, A, and E; the cyclin-dependent kinase inhibitors p18, p19, and p27; CDK2, CDK6, E2F-4, E2F-5, and DP-1. Also, the expression profile of the *NF2* gene was included in the analysis.

Table III summarizes the results for the expression profiles of the 14 genes in the pRb-CDK pathway in VS compared with the normal vestibular nerve. The Rb-1 gene was underexpressed in two tumors (VS5 and VS8). Cyclin D1 was overexpressed in two tumors and underexpressed in one other tumor. Cyclin D2 and CDK6 were underexpressed in one VS each. No significant change in cyclin A expression was found in all eight tumors analyzed. Intriguingly, CDK2 was highly underexpressed in seven of the eight tumors studied. The only tumor that did not show CDK2 downregulation was VS8; however, this cystic tumor displayed underexpression of cyclin E and overexpression of CDK6. The VS8 tumor from a 19-year-old man was the largest schwannoma (approximately 3 × 3 cm) from the group of tumors studied.

Of the three cyclin-dependent kinase inhibitors analyzed, only p27 was found to be underexpressed in three VS tumors including the VS8 tumor. It should be mentioned that all these three tumors were from young patients with large tumors (VS5, VS7, and VS8), and their ages were 39, 28, and 19 years, respectively. Each tumor had a diameter greater than 2.5 cm. These results suggest that these vestibular schwannomas may have had a faster growth rate compared with the other tumors when taking the size and patient age into consideration.

The transcription factor E2F-4 was underexpressed in two tumors including the VS5 that also had Rb-1 downregulation. No significant change in E2F-5 expression was seen for all eight VS tumors studied. Interestingly, the DP-1 protein, which associates with E2F, was underexpressed in six of the eight tumors. As a comparison, the *NF2* gene was underexpressed in three tumors and the others showed no significant difference in expression.

TABLE III.
Differential Gene Expression of the pRb-CDK Pathway in Vestibular Schwannomas Compared With the Normal Vestibular Nerve.*

	VS 1-C	VS 2-C	VS 3-S	VS 4-S	VS 5-S	VS 6-NF2	VS 7-S	VS 8-C
Rb-1	−1.23	−1.08	1.06	−1.23	**3.44**	2.56	2.05	**5.79**
CDK6	1.91	1.46	−1.50	1.06	−1.60	−1.06	−2.58	**−3.90**
CDK2	**7.12**	25.38	14.41	31.14	11.39	15.84	3.53	1.83
Cyclin D1	−2.92	**−6.53**	−2.28	−2.15	−2.14	**−6.13**	8.36	2.78
Cyclin D2	**3.08**	2.20	−1.44	−1.87	1.76	1.86	−1.51	−1.48
Cyclin E	1.14	1.17	1.18	−1.41	2.37	2.77	1.49	**4.72**
Cyclin A	1.50	−1.11	−1.02	−2.46	2.13	1.58	1.41	2.03
P18	−1.35	1.00	1.00	−1.89	2.78	2.15	1.29	2.28
P19	2.21	1.56	1.06	1.12	2.12	1.62	−1.14	−1.62
P27	1.24	1.17	2.02	1.76	**3.67**	1.75	**4.58**	7.82
DP-1	**4.39**	16.8	4.88	7.35	5.18	8.15	−1.65	1.43
E2F-4	1.73	1.40	1.74	1.00	**3.18**	3.34	2.71	1.75
E2F-5	−1.25	−2.15	−1.44	−1.84	**3.42**	2.70	1.21	−1.05
NF2	−1.81	−2.81	1.22	−1.53	**3.79**	3.45	3.76	2.30

*Significantly under- or overexpressed genes are shown in bold. A three-fold or greater difference is considered significant. Genes under expressed in the VS tissues are shown as positive values while those overexpressed have a negative value.

S = sporadic tumor; C = cystic tumor; NF2 = neurofibromatosis type 2.

TABLE IV.
Analysis of CDK2 Expression in Vestibular Schwannomas by Real-time PCR.*

Patient	CDK2 (C_T)
VS1	22.6
VS2	171.3
VS3	243.9
VS4	163.1
VS5	156.5
VS6	98.4
VS7	N/A
VS8	−4.3

*Genes underexpressed in the VS tissues are shown as positive values.
N/A = insufficient quantity of RNA available to perform real-time PCR analysis; C_T = comparative cycle threshold.

Real-Time Polymerase Chain Reaction Analysis

The real-time PCR technique is a sensitive method to quantify gene expression and was used to confirm the expression data from the cDNA microarray analysis. CDK2, which was underexpressed in six of the seven VS tumors examined, was further analyzed. Table IV summarizes the real-time PCR data. Consistent with the cDNA microarray results, the real-time PCR analysis also showed CDK2 underexpression in the same seven tumors compared with the normal vestibular nerve (compare Table III with Table IV). However, the overall reduction in CDK2 expression was greater from real-time PCR than from cDNA microarray analysis. Although CDK2 expression in VS8 appeared to be similar to that in normal vestibular nerve, a slightly greater CDK2 expression was detected using real-time PCR. Tumor VS7 had insufficient RNA to perform real-time PCR.

Immunohistochemical Analysis

To examine the CDK2 protein expression, immunohistochemical analysis was performed on VS tissue sections using an anti-CDK2 antibody. A representative CDK2 staining pattern is shown in Figure 2. The anti-CDK2 immunoreactivity could be seen primarily localized in the vestibular nerve along the surface of the tumor. Schwann cells, axons, and ganglion cells had high anti-CDK2 activity. In contrast, the palisading spindle-shaped schwannoma tumor cells showed only weak CDK2 staining. Normal cranial nerve was also stained with anti-CDK2 antibody and showed staining similar to the vestibular nerve alongside the tumor (Fig. 2).

Fig. 2. Immunohistochemical staining of schwannoma with an anti–cyclin-dependent kinase type 2 (anti-CDK2) antibody. Note the prominent uptake in the vestibular nerve (VN) along the surface of the tumor (T). The arrow points to Schwann cells that show high anti-CDK2 activity.

DISCUSSION

In addition to mutations in oncogenes and tumor suppressor genes, deregulated expression of growth regulatory genes frequently occurs in human tumors. Growth regulatory gene products are responsible for cellular proliferation. Alteration of expression of these genes results in abnormal cell growth, leading to tumor formation. Consistent with this notion, our study shows that the pRb-CDK pathway is frequently deregulated in vestibular schwannomas.

Mutations in the *NF2* gene are frequently found in vestibular schwannomas. We also identified mutations in the coding region of the *NF2* gene in six of the eight tumors studied (data not shown). In addition, *NF2* was significantly underexpressed in three of the eight schwannomas studied, suggesting that, although NF2 RNA could be detected, mutations in the *NF2* gene may cause instability in the *NF2* protein as reported previously.[5] However, how the NF2 protein is linked to the growth regulatory pathway is presently not understood. The fact that expression of the pRb-CDK pathway genes is altered in all vestibular schwannomas studied suggests that this pathway is a potential downstream target.

Alterations in Rb-1 expression may result in loss of pRb tumor suppressor function and have been reported in lung cancer,[14,15] osteogenic sarcomas,[16] leukemias,[17] prostate,[18] and bladder cancer.[19] Furthermore, loss of both Rb alleles leads to retinoblastoma formation. Curiously, we also found that two of the eight vestibular schwannomas studied showed downregulation of the Rb-1 gene.

Cyclin D1 overexpression has been reported in breast, colon, lung, liver, gastric, head and neck, and bladder cancer.[7] Overexpression of cyclin D2 and CDK4 has been identified in testicular germ cell tumors.[20] Also, CDK6 upregulation has been reported in some gliomas,[21] and its downregulation has been observed in the majority of testicular seminomas.[20] Intriguingly, we observed CDK6 overexpression and cyclin E underexpression in a large cystic tumor when compared with the normal vestibular nerve. Most important, both the CDK2 RNA and protein are underexpressed in the rest of seven vestibular schwannomas analyzed. Decreased CDK2 and cyclin E expression have been previously reported in testicular germ cell tumors.[20] In contrast, CDK2 and cyclin E expression have been reported to be high in human cutaneous melanomas and their metastases when compared with other cyclin-dependent kinases and cyclins.[22] Furthermore, cyclin E overexpression has been used to predict the presence of metastasis with prognostic value in non-small

cell lung cancer.[23] Also, overexpression of CDK2 has been observed for oral and laryngeal squamous cell carcinoma.[24,25] In addition, proliferating Schwann cells established in culture from rat sciatic nerves showed higher CDK2 protein expression than nonproliferating cells.[26] Taken together, our results suggest that decreased expression of CDK2 in vestibular schwannomas may relate to the slow growth rate of the tumor.

The cyclin-dependent kinase inhibitor p27 has been assigned to chromosome 12p12, a region often mutated in childhood acute lymphoblastic leukemia (ALL).[27] Consistently, we found that three vestibular schwannomas showed p27 downregulation. In contrast, overexpression of p27 in non-Hodgkin's lymphoma has been shown to be of prognostic value.[28] Other authors have also reported that high expression of p27 and inhibition of cyclin E/CDK2 may favor survival of small cell lung carcinoma cells by preventing apoptosis in an environment that is not favorable for cell proliferation.[29]

The Rb-related protein p130 can be phosphorylated in a CDK2 dependent fashion resulting in the activation of E2F-4 and E2F-5 transcription factors.[30] It is likely that deregulation of E2F transcription factors would interrupt normal cell cycle progression. DP-1 associates with E2F transcription factors. The functional significance of underexpression of E2F-4, E2F-5, and their associated protein DP-1 in some vestibular schwannomas is currently unknown.

One important consideration that should be pointed out in our analysis is the use of the paired normal vestibular nerve as the control tissue. Experiments using cultured Schwann cells may yield data for direct comparison with schwannoma tissue. However, the drawback of using such a cell culture is that culture conditions could alter gene expression patterns. Studies with colon cancer have revealed that normal colon tissue, colon carcinoma tissue, and colon carcinoma cell culture yield distinctly different expression profiles.[10] In addition, limited division, as well as growth arrest, has been commonly observed with cultured human VS cells, and a stable human Schwann cell line has not been established. Nevertheless, a direct comparison of protein expression (CDK2) in tissue section, together with quantitative real-time PCR analysis, yielded results consistent with cDNA microarray data when schwannoma tumors were compared with the normal vestibular nerve.

CONCLUSION

Approximately 0.5% of genes expressed in vestibular schwannomas showed deregulation compared with those in the normal vestibular nerve. Among deregulated genes, several members of the pRb-CDK pathway, including Rb-1; cyclin D1, D2, and E; and CDK2, CDK6, p27, E2F, and DP-1, were found. In particular, CDK2 expression was downregulated in seven of the eight vestibular schwannomas studied. Further collaboration with other centers and investigation in vitro and in vivo into the regulatory mechanisms governing CDK2 expression may lead to a better understanding of VS tumorigenesis.

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