Immunohistochemical analysis of insulin-like growth factor 1 and its receptor in sporadic schwannoma/ peripheral nerve sheath tumour

Fumiaki Matsubara[1], Takuyuki Katabami[2],
Shiko Asai[2], Yasushi Ariizumi[3], Ichiro Maeda[3],
Masayuki Takagi[3], May McNamara Keely[4],
Katsuhiko Ono[4], Takashi Maekawa[4],
Yasuhiro Nakamura[4], Yasushi Tanaka[1] and
Hironobu Sasano[4]

Abstract

Objective: To investigate the immunohistochemical localization of insulin-like growth factor 1 (IGF-1) and IGF-1 receptor (IGF-1R) in archival specimens of sporadic schwannoma.

Method: This study retrospectively analysed the immunolocalization of IGF-1 and IGF-1R in schwannoma specimens collected from all patients with sporadic schwannoma that were treated by two institutions in Japan. The study also evaluated the association between the extent of the IGF-1 and IGF-1R immunoreactivity and several clinicopathological characteristics (age, sex and maximum tumour dimension).

Results: The study examined a total of 29 sporadic schwannoma specimens. IGF-1 and IGF-1R immunoreactivity was detected in the majority of the specimens regardless of their anatomical location. IGF-1 and IGF-1R were not co-localized. There was no association between the extent of the IGF-1 and IGF-1R immunoreactivity and the clinicopathological characteristics of the patients.

[1]Department of Internal Medicine, Division of Metabolism and Endocrinology, St Marianna University School of Medicine, Kawasaki, Kanagawa, Japan
[2]Department of Internal Medicine, Division of Metabolism and Endocrinology, St Marianna University School of Medicine Yokohama City Seibu Hospital, Yokohama, Kanagawa, Japan
[3]Department of Pathology, St Marianna University School of Medicine, Kawasaki, Kanagawa, Japan

[4]Department of Anatomical Pathology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan

Corresponding author:
Takuyuki Katabami, Department of Internal Medicine, Division of Metabolism and Endocrinology, St Marianna University School of Medicine, Yokohama City Seibu Hospital, 1197-1 Yasashi-cho, Asahi-ku, Yokohama city, Kanagawa 241-0811, Japan.
Email: t2kataba@marianna-u.ac.jp

Conclusions: As IGF-1 and IGF-1R immunoreactivity was detected in the majority of sporadic schwannoma specimens regardless of their anatomical location, these findings suggest that an IGF-1/IGF-1R loop could play a role in the tumorigenesis and progression of schwannomas via an autocrine–paracrine mechanism.

Introduction

Schwannomas or peripheral nerve sheath tumours are associated with abnormalities of the neurofibromin 2 (merlin) (*NF2*) gene that encodes the tumour-suppressor protein merlin.[1] Insulin-like growth factor 1 receptor (IGF-1R) was markedly overexpressed in primary schwannomas occurring in patients with neurofibromatosis type 2 (NF2).[2] In addition, in these NF2 patients, insulin-like growth factor 1 (IGF-1) was upregulated and released into the circulation from the merlin-deficient schwannoma tumour cells, suggesting that an IGF-1 and IGF-1R autocrine–paracrine loop could play a role in the development of these tumours.[2] A patient with sporadic retroperitoneal schwannoma or peripheral nerve sheath tumour harbouring upregulated IGF-1 and IGF-1R levels was recently described.[3] However, other than vestibular schwannoma,[4] this IGF-1/IGF-1R autocrine–paracrine loop has not been explored in the wider spectrum of sporadic schwannoma. Therefore, this study investigated the immunohistochemical localization of IGF-1 and IGF-1R in patients with sporadic schwannoma. It also evaluated the association between the status of the IGF-1 and IGF-1R immunoreactivity and several clinicopathological characteristics of the patients in order to further explore its significance.

Patients and methods

Patients

This retrospective study analysed archival tumour specimens from all patients with benign sporadic schwannoma who underwent surgery at the Department of Internal Medicine, Division of Metabolism and Endocrinology, St Marianna University School of Medicine, Kawasaki, Kanagawa, Japan or the Department of Anatomical Pathology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan between August 2014 and March 2015. Patients with a diagnosis of neurofibromatosis were excluded. As a result of the previous immunohistochemical investigation of a 63-year-old woman with a retroperitoneal schwannoma and upregulation of both IGF-1 and IGF-1R (previously published in Japanese, data not shown),[3] this present study aimed to examine as many schwannomas as possible that originated from the retroperitoneum. As a consequence, the distribution of the tumour site in this present study varied compared with a previous report.[5] The clinicopathological characteristics of age, sex, maximum tumour dimension and site of tumour occurrence were selected and data were retrieved from the pathological records.

The study protocol was approved by the Ethical Review Boards of St Marianna University School of Medicine (no. 2724)

and Tohoku University Graduate School of Medicine (no. 2014-1-416). Informed consent was not required from the patients that provided archival schwannoma specimens and demographic and clinical data as these were used in an anonymized manner.

Tissue preparation and immunohistochemistry

Paraffin-embedded schwannoma specimens were retrieved from the surgical pathology files at St Marianna University School of Medicine and Tohoku University Graduate School of Medicine. The following methodology was used as reported previously.[6] Tissues were cut into small pieces, fixed in 10% formalin, processed, and embedded in paraffin. Sections were cut at 5–6 μm thickness, deparaffinized, and pretreated with 10 mM citrate buffer (pH 6.0) in an autoclave (high pressure steam sterilizer LSX500; TOMY, Tokyo, Japan) for 5 min at 121°C for IGF-1 immunostaining and in a microwave oven (DR-4215; Twinbird, Tsubame City, Japan) for 20 min at 100°C for IGF-1R immunostaining, in order to activate the antigenicity masked by aldehyde fixation. After washing with 0.01 M phosphate-buffered saline (PBS; pH 7.4) three times every 5 min, the specimens were treated with 10% normal goat serum (Nichirei Biosciences, Tokyo, Japan) for 30 min at room temperature. Immunohistochemical procedures were performed using the streptavidin-biotin-peroxidase method with a Histofine Immunostaining Kit (Nichirei Biosciences). The sections were incubated for 18 h at 4°C in a humidified chamber with the following rabbit antihuman polyclonal antibodies: recombinant human IGF-1 (dilution 1:100; Abcam, Cambridge, UK) and human IGF-1R (dilution 1:100; Abcam). After being rinsed three times in 0.01 M PBS (pH 7.4), the sections were submerged in a bath of 0.3% hydrogen peroxide in 100% methanol for 30 min to block the endogenous peroxidase activity. They were then incubated at room temperature in a humidified chamber with biotinylated goat antirabbit immunoglobulin and peroxidase-conjugated streptavidin from the Histofine Immunostaining Kit for 30 min each. After further rinsing three times in 0.01 M PBS (PH 7.4), the sections were immersed for 5 min in a solution containing 30 mg 3,3′-diaminobenzidine (DAB)/6 ml 0.05 M Tris buffer (pH 7.6), which was cooled to −20°C, 80 ml distilled water, 20 ml 0.05 M Tris buffer (pH 7.6), and 20 μl 30% hydrogen peroxidase at room temperature. Following DAB staining, the sections were counterstained with haematoxylin. Paraffin-embedded tissue sections from segment six of the human liver from an autopsy case were provided by the Department of Anatomical Pathology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan and were used as positive controls for the immunostaining. For negative control slides, 0.01 M PBS (pH 7.4) was used instead of the primary antibody on a schwannoma specimen from one of the patients.

Immunohistochemical evaluation

Immunohistochemical staining was evaluated by three authors (F.M., Y.N. and H.S.) under a light microscope (model BX51; Olympus, Tokyo, Japan) in a semi-quantitative manner. Photographic images were captured using a digital camera (model DP26®; Olympus). Five fields of view at ×200 magnification were examined and 100 cells were counted in total per specimen. The relative immunostaining intensity in the nucleus and cytoplasm of tumour cells was scored as follows: (i) negative (−), when there was no immunohistochemical staining present; (ii) weakly positive (+), when the immunostaining intensity of all of the

positive cells was weak or when the proportion of strongly stained cells was <10%; and (iii) markedly positive (++), when the proportion of strongly positive cells was ≥10%.

Statistical analyses

All statistical analyses were performed with EZR (Saitama Medical Centre, Jichi Medical University, Saitama, Japan), a graphical user interface for R (the R Foundation for Statistical Computing, Vienna, Austria), which is a modified version of R commander designed to add statistical functions frequently used in biostatistics.[7] Data are presented as mean ± SE or *n* of patients. Statistical analyses were undertaken using the Mann–Whitney *U*-test or χ^2-test. A *P*-value < 0.05 was considered statistically significant.

Results

Archival schwannoma specimens from 29 patients were examined during this study. The demographic and clinical characteristics of the patients are presented in Table 1.

Representative photomicrographs of the immunohistochemical staining and the scoring system used are shown in Figure 1. The schwannoma specimen from patient number 11 was also used as the negative control in which 0.01 M PBS (pH 7.4) was used instead of the primary antibody. A representative image confirming the positive immunoreactivity of the schwannoma specimen from patient number 11 is shown in Figure 1. Representative photomicrographs of the immunohistochemical staining of the positive and negative controls are shown in Figure 2.

The semi-quantitative results of the immunohistochemical staining are summarized in Tables 2 and 3. All schwannoma tissues demonstrated both nuclear and cytoplasmic immunoreactivity of IGF-1 and the status of its localization tended to be

Table 1. Demographic and clinical characteristics of patients (*n* = 29) with schwannomas who were included in this study to investigate the immunohistochemical localization of insulin-like growth factor 1 and insulin-like growth factor 1 receptor.

Characteristic	Patients with schwannomas *n* = 29
Sex, male/female	16/13
Age, years	57.6 ± 2.8
Maximum tumour dimension, cm	3.4 ± 0.5[a]
Site of tumour occurrence	
Intracranial	3 (10.3)
Cervical, limbs	11 (37.9)
Spinal	2 (6.9)
Mediastinum	2 (6.9)
Thoracic wall	1 (3.4)
Retroperitoneum	10 (34.5)

Data presented as mean ± SE or *n* of patients (%).
[a]Unknown for six patients.

stronger in the nuclei than in the cytoplasm. Within the semi-quantitative grading system used in the study, nuclear immunointensity was (+) in nine patients (31%) and (++) in 20 patients (69%), whereas cytoplasmic immunointensity was (+) in 26 patients (90%) and (++) in three patients (10%). In 19 patients, there were discrepancies between the nuclear and cytoplasmic immunointensities of IGF-1 staining patterns, with a majority of these demonstrating weaker cytoplasmic than nuclear intensity.

In contrast to the IGF-1, a number of tissues lacked nuclear and/or cytoplasmic immunoreactivity for IGF-1R, and staining tended to be more marked in the cytoplasm than in the nuclei. IGF-1R immunostaining intensity in the nuclei was (−) in 20 patients (69%), (+) in nine patients (31%), and no patients demonstrated (++) immunoreactivity. In the cytoplasm, six patients (21%) were (−), 21 patients (72%) were (+), and two patients (7%) were (++). In 14 patients, there were discrepancies between the nuclear and cytoplasmic immunointensity
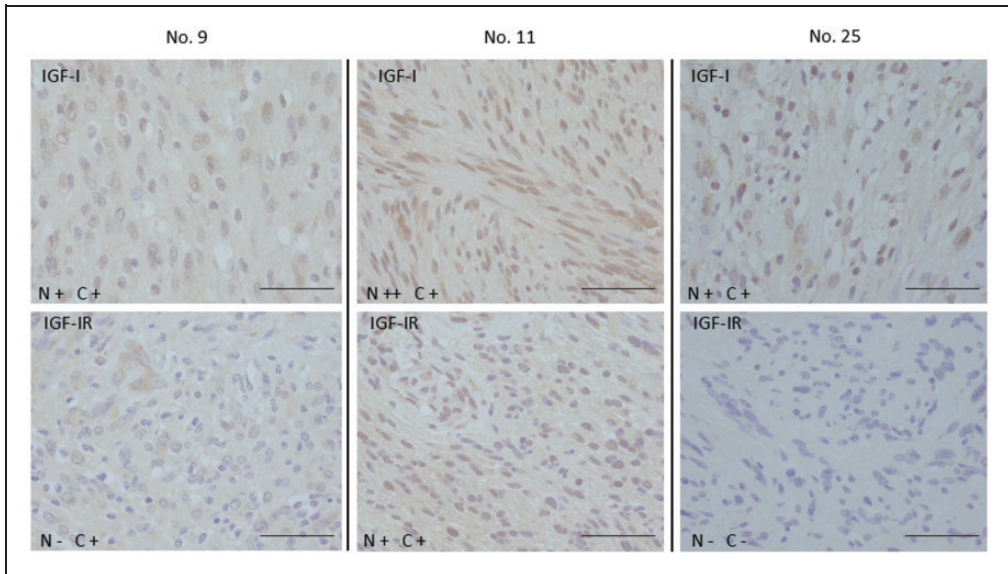
Figure 1. Representative photomicrographs showing the insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 1 receptor (IGF-1R) immunoreactivity in schwannomas with examples of the scoring system. The schwannoma specimen from patient number 11 was also used as the negative control sample in which 0.01 M phosphate-buffered saline (pH 7.4) was used instead of primary antibody. No., patient number; N, nucleus; C, cytoplasm. Scale bar 50 μm.

of IGF-1R staining, with a majority of these showing more marked cytoplasmic than nuclear immunoreactivity. When examining the relationship between the localization of IGF-1 and IGF-1R, these two proteins were not found to be co-localized.

The results of the analysis of whether three clinicopathological characteristics (age, sex and maximum tumour dimension) varied between subgroups stratified according to the level of IGF-1 and IGF-1R immunostaining in all 29 patients are summarized in Tables 4 and 5. There were no significant differences in age and the maximum tumour dimension between each group for nuclear and cytoplasmic IGF-1 and IGF-1R immunoreactivity (Mann–Whitney U-test). In addition, the frequencies of IGF-1 and IGF-1R immunoreactivity in the nucleus and cytoplasm were not significantly associated with sex distribution (χ^2-test).

Discussion

Schwannomas or peripheral nerve sheath tumours are rare tumours that originate from neural crest cells.[8] They are usually interpreted as slow-growing benign nerve sheath tumours that are most frequently associated with NF2, a hereditary disease caused by the loss of the *NF2* gene encoding the tumour-suppressor protein merlin.[1] Merlin is considered to control the number of adhesion and growth factor receptors at the cell surface of Schwann cells by inhibiting their delivery to the plasma membrane;[9] thus, the loss of merlin in NF2 leads to the increased delivery of adhesion and growth factor receptors to the cell surface. Previous research has examined the role of the IGF-1/IGF-1R signalling pathway in the pathogenesis of hereditary schwannoma,[2] but this pathway has not been reported in sporadic schwannomas other than vestibular types.[4]
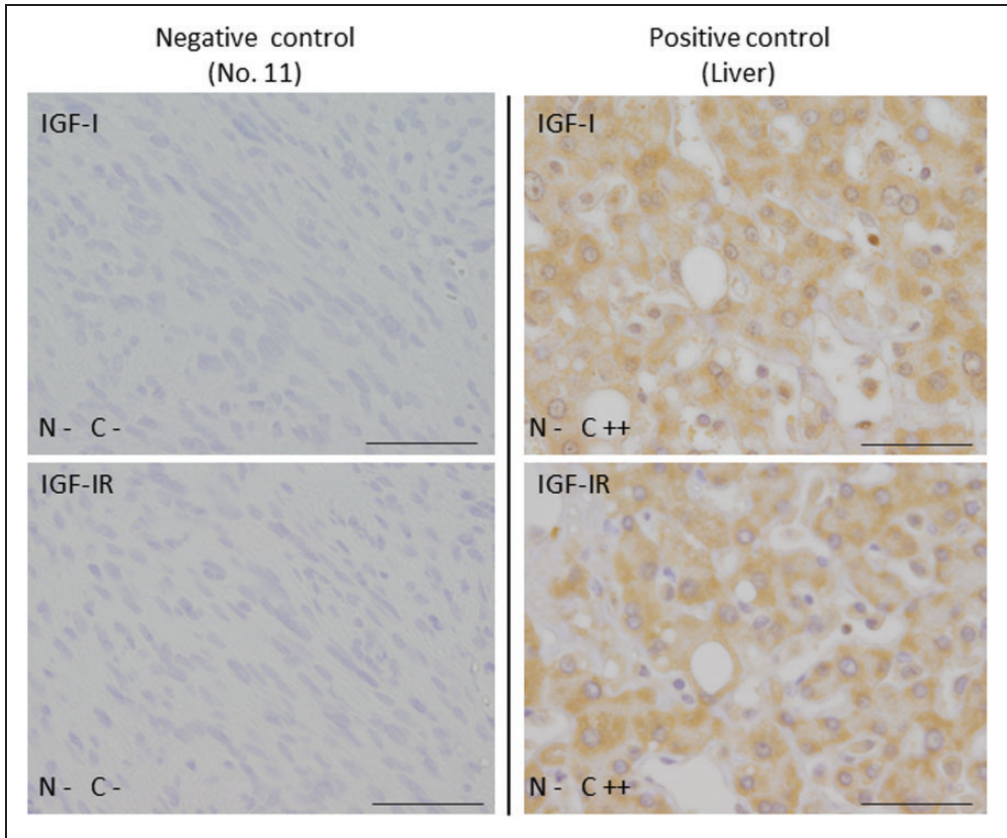
Figure 2. Representative photomicrographs showing the insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 1 receptor (IGF-1R) immunoreactivity in the schwannoma specimen from patient number 11 and human liver (positive control tissue). IGF-1 was detected mainly in the cytoplasm and partly in the perinuclear area, whereas IGF-1R was detected only in cytoplasm in the human liver. A nonspecific response was not found in the negative control patient number 11 (No. 11) when 0.01 M phosphate-buffered saline (pH 7.4) was used instead of primary antibody. No., patient number; N, nucleus; C, cytoplasm. Scale bar 50 μm.

Therefore, this present study investigated the immunohistochemical localization of IGF-1 and IGF-1R in sporadic schwannoma specimens.

In this current study, the majority of sporadic schwannoma specimens expressed IGF-1 and IGF-1R, suggesting that these proteins might contribute to tumorigenesis and/or progression in sporadic as well as in hereditary cases. An investigation of the expression of IGF-1R in what were classified as benign mesenchymal schwannomas found that none of the eight specimens showed any positivity.[10] The difference between the results of the current study and those of this previous report[10] suggests that further investigations are required for clarification. It is also difficult to interpret the pathophysiological significance of the current results because IGF-1 and IGF-1R upregulation occurs in many types of human tumours along with their ubiquitous expression in various normal tissues.[11] There are few previous reports examining these signalling molecules in Schwann cells and human schwannomas. For example, a previous

Table 2. Results of insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 1 receptor (IGF-1R) immunohistochemical staining of retroperitoneal schwannomas ($n = 10$).

Patient number	Age	Sex	Site of tumour occurrence	Tumour dimensions, cm	IGF-1 Nucleus	IGF-1 Cytoplasm	IGF-1R Nucleus	IGF-1R Cytoplasm
1	73	F	Interior of the pelvis	6.0 × 5.0 × 3.6	++	+	−	+
2	39	M	Interior of the pelvis	5.5 × 3.5	++	+	−	+
3	53	M	Between the aorta and left kidney	3.0 × 3.5	++	+	+	+
4	57	F	Near the left kidney	Unknown	+	++	−	++
5	67	M	Interior of the pelvis (between the sacrum and rectum)	10.0 × 10.0	+	+	−	−
6	57	F	Interior of the pelvis	4.0 × 3.5	++	+	−	+
7	63	F	Interior of the pelvis	Unknown	++	+	−	++
8	59	M	Near the hepatoduodenal ligament	9.3 × 6.3 × 3.5	+	+	−	+
9	63	F	Near the left adrenal gland	5.5 × 4.7 × 2.0	+	+	−	+
10	49	F	Interior of the pelvis (complicated with myoma)	4.0 × 3.5 × 3.0	++	+	+	+

F, female; M, male.

report described the overexpression and release of IGF-1 in primary schwannoma cells from NF2 patients and that downstream pathways of these molecules could regulate cell proliferation, cell–matrix adhesion, and survival of these cells possibly through an autocrine/paracrine mechanism.[2] In addition, another study reported that the transcriptome analysis for vestibular schwannomas in sporadic patients, patients with a history of NF2, and those with normal vestibular tissues identified tumour-specific changes.[4] Upregulation of IGF-1 signalling was detected in both sporadic and germline tissues.[4] Of particular interest, no specific difference in gene expression was detected between sporadic and germline vestibular schwannomas, suggesting that they could share similar biological aetiology and dependence on the upregulation of IGF-1 signalling.[4] Therefore, the results presented by this current study combined with those of a previous report could provide solid support for IGF-1 signalling as an underlying mechanism in the development of schwannomas.[4] As in a previous report,[2] IGF-1 was detected in the cytoplasm and nuclei of schwannoma cells in the present study, whereas IGF-1R immunoreactivity in these cells was consistently detected in the cytoplasm with variations in nuclear immunointensity among specimens. At present, the explanation for this variation in immunoreactivity is not known, although previous studies correlated increased immunoreactivity of IGF-1R with increased tumorigenicity.[2] A previous study reported that an isoform of human IGF-1 was localized to the nuclei, particularly to the nucleolus.[12] Nuclear and nucleolar localization also occurred when the mature IGF-1 domain was deleted from chimeras, or when signal peptides were deleted.[12] However it is not known at this juncture

Table 3. Results of insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 1 receptor (IGF-1R) immunohistochemical staining of schwannomas other than retroperitoneal cases ($n = 19$).

Patient number	Age	Sex	Site of tumour occurrence	Tumour dimensions, cm	IGF-1		IGF-1R	
					Nucleus	Cytoplasm	Nucleus	Cytoplasm
11	76	M	Left cerebellopontine angle	Unknown	++	+	+	+
12	59	F	Left lower leg	1.8 × 2.0 × 1.0	++	+	−	+
13	32	M	Right femur	3.0 × 2.5 × 1.9	++	++	−	+
14	48	M	Back of the right hand	2.1 × 1.8 × 1.3	++	+	−	+
15	70	F	Right thumb	Unknown	++	+	+	+
16	65	F	Extramedullary tumour of the thoracic spinal cord	2.2 × 2.0 × 1.4	++	+	+	+
17	76	M	Right acoustic nerve	Unknown	++	+	−	−
18	48	M	Spinal arachnoid cyst	4.1 × 2.1 × 0.8	++	++	−	+
19	69	M	Mediastinum (bronchial bifurcation)	Unknown	+	+	−	+
20	59	F	Right acoustic nerve	1.1 × 1.4 × 0.7	+	+	−	+
21	70	M	Subcutaneous	4.0 × 2.7 × 2.4	+	+	−	−
22	47	F	Right digitus annularis	1.3 × 1.0 × 0.7	++	+	+	+
23	46	M	Right palm	3.1 × 2.0 × 2.0	+	+	−	−
24	45	M	Mediastinum (right side of the fourth thoracic vertebra)	2.0 × 1.0 × 0.8	++	+	−	−
25	18	M	Chest wall tumour (left fifth intercostal)	2.4	+	+	−	−
26	72	F	Right lower leg	1.3 × 1.8 × 1.5	++	+	+	+
27	83	M	Left cervical	2.5 × 1.5 × 2.0	++	+	+	+
28	39	F	Cervical	2.0 × 1.5 × 1.5	++	+	−	+
29	69	M	Left front arm	2.1 × 1.3 × 1.1	++	+	+	+

M, male; F, female.

how an isoform of IGF-1 could affect any of these nucleolar functions of the cells. Despite the similarity in the gene expression profile between sporadic and germline vestibular schwannomas described above, not all sporadic schwannomas represent *NF2* inactivation.[13] In addition, there are some differences in the allelic changes of the *NF2* gene or the chromatin remodelling gene (*SMARCBA1*) between NF2-derived and sporadic schwannomas.[14] Thus far, the genotype–phenotype correlation has not been determined, but it is possible that the underlying genetic variance in the disease could contribute to differential involvement of IGF signalling pathways, a possibility that requires further research.

In other tumour types, variation in the role of the IGF-1/IGF-1R pathways and their correlation with clinicopathological characteristics is equally well documented. For example, nuclear localization of IGF-1R was identified in multiple malignant and nonmalignant epithelial cell lines, in a substantial part of the clear-cell renal carcinoma, and also in a small cohort of sarcomas.[15,16] Another report demonstrated that nuclear IGF-1R was associated with an

Table 4. Comparison of clinicopathological characteristics between two subgroups of patients with different levels of insulin-like growth factor 1 (IGF-1) immunohistochemical staining of their schwannomas.

	IGF-1 staining intensity in the nucleus	
Clinicopathological characteristic	(+) $n = 9$	(++) $n = 20$
Sex, male/female	6/3	10/10
Age, years	56.4 ± 5.4	58.5 ± 3.3
Maximum tumour dimension, cm	5.1 ± 1.3	3.2 ± 0.4
	IGF-1 staining intensity in the cytoplasm	
Clinicopathological characteristic	(+) $n = 26$	(++) $n = 3$
Sex, male/female	14/12	2/1
Age, years	59.3 ± 2.8	45.7 ± 7.3
Maximum tumour dimension, cm	3.7 ± 0.5	3.5 ± 0.6

Data presented as mean $\pm$ SE or n of patients.
No significant between-group differences (P $\geq$ 0.05); Mann–Whitney U-test or χ^2-test.

Table 5. Comparison of clinicopathological characteristics between two subgroups of patients with different levels of insulin-like growth factor 1 receptor (IGF-1R) immunohistochemical staining of their schwannomas.

	IGF-1R staining intensity in the nucleus	
Clinicopathological characteristic	(−) $n = 20$	(+) $n = 9$
Sex, male/female	12/8	4/5
Age, years	54.3 ± 3.3	64.8 ± 4.2
Maximum tumour dimension, cm	4.1 ± 0.6	2.1 ± 0.4
	IGF-1R staining intensity in the cytoplasm	
Clinicopathological characteristic	(−) $n = 6$	(+) or (++) $n = 23$
Sex, male/female	6/0	10/13
Age, years	53.6 ± 8.8	58.7 ± 2.7
Maximum tumour dimension, cm	4.3 ± 1.5	3.3 ± 0.5

Data presented as mean $\pm$ SE or n of patients.
No significant between-group differences (P $\geq$ 0.05); Mann–Whitney U-test or χ^2-test.

adverse prognosis in embryonal rhabdomyosarcoma.[17] The analysis of the correlation between the clinicopathological characteristics and IGF-1/IGF-R immunoreactivity studies in breast,[18,19] thyroid,[20] and renal cancer[21] failed to detect an interaction between IGF-1/IGF-R and tumour size. A significant correlation was reported between IGF-1R and insulin receptor substrate-1 downregulation with tumour progression in advanced human breast cancer,[18] whereas IGF-1 immunoreactivity in human breast cancer was associated with a more differentiated type of epithelial cell.[19]

This phenomenon of increasing dedifferentiation correlated with a decreased IGF-1 content suggests a potential role for this factor in tumour progression. All of these studies suggest that IGF-1/IGF-1R pathways are active in cancers,[15–21] but further investigations are required in order to fully understand their clinical and biological significance.

An important question that should also be considered with regard to the role of IGF pathways in schwannomas is the relative contribution of circulating levels of IGF ligands as compared with localized production. Because of the retrospective nature of this current analysis, it was not possible to measure serum IGF-1 and its binding protein concentration in the patients that were examined. In addition, previous studies have reported the involvement of IGF-1 and IGF-1R signalling in the pathogenesis of schwannoma,[1,2,4] whereas circulating IGF-1 levels have not been reported in patients with schwannoma. As incidental evidence, an increased risk of schwannoma has not been reported in patients with acromegaly,[22] suggesting that IGF-1 concentrations around the tumour and/or intratumoral IGF-1 signalling as opposed to systemic IGF-1 concentrations could be important for oncogenesis in the majority of schwannomas, but further investigations are required for clarification.

The present study had several limitations. First, the sample size was small despite every effort being made to collect as many relevant cases as possible from both institutions. As a consequence, it is possible that the results that were obtained might not represent the general features or characteristics of schwannoma. Secondly, it was not possible to perform an absorption test of the primary antibodies in order to confirm the specificity of the immunoreactivity obtained in this study due to the lack of availability of the corresponding antigens.

In conclusion, IGF-1 and IGF-1R immunoreactivity was detected in the majority of sporadic schwannoma specimens regardless of their anatomical location. These current findings suggest that an IGF-1/IGF-1R loop could play a role in the tumorigenesis and progression of schwannomas via an autocrine–paracrine mechanism.

References

1. Ammoun S and Hanemann CO. Emerging therapeutic targets in schwannomas and other merlin-deficient tumors. *Nat Rev Neurol* 2011; 7: 392–399.
2. Ammoun S, Schmid MC, Ristic N, et al. The role of insulin-like growth factors signaling in merlin-deficient human schwannomas. *Glia* 2012; 60: 1721–1733.
3. Matsubara F, Katabami T, Matsuba R, et al. Retroperitoneal schwannoma with overexpression of IGF-1 and -IR associated with acromegaly and papillary thyroid carcinoma: a case report. *ACTH Related Peptides* 2014; 25: 50–53. (In Japanese).
4. Agnihotri S, Gugel I, Remke M, et al. Gene-expression profiling elucidates molecular signaling networks that can be therapeutically targeted in vestibular schwannoma. *J Neurosurg* 2014; 121: 1434–1445.
5. Toutouzas KG, Tsamis D, Kekis PB, et al. Laparoscopic resection of an adrenal schwannoma. *JSLS* 2012; 16: 663–667.

6. Miki Y, Nakata T, Suzuki T, et al. Systemic distribution of steroid sulfatase and estrogen sulfotransferase in human adult and fetal tissues. *J Clin Endocrinol Metab* 2002; 87: 5760–5768.

7. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013; 48: 452–458.

8. Târcoveanu E, Dimofte G, Bradea C, et al. Adrenal schwannoma. *JSLS* 2009; 13: 116–119.

9. Lallemand D, Manent J, Couvelard A, et al. Merlin regulates transmembrane receptor accumulation and signaling at the plasma membrane in primary mouse Schwann cells and in human schwannomas. *Oncogene* 2009; 28: 854–865.

10. Lasota J, Wang Z, Kim SY, et al. Expression of the receptor for type i insulin-like growth factor (IGF1R) in gastrointestinal stromal tumors: an immunohistochemical study of 1078 cases with diagnostic and therapeutic implications. *Am J Surg Pathol* 2013; 37: 114–119.

11. Hartog H, Wesseling J, Boezen HM, et al. The insulin-like growth factor 1 receptor in cancer: old focus, new future. *Eur J Cancer* 2007; 43: 1895–1904.

12. Tan DS, Cook A and Chew SL. Nucleolar localization of an isoform of the IGF-I precursor. *BMC Cell Biol* 2002; 3: 17.

13. Jacoby LB, MacCollin M, Louis DN, et al. Exon scanning for mutation of the NF2 gene in schwannomas. *Hum Mol Genet* 1994; 3: 413–419.

14. Hilton DA and Hanemann CO. Schwannomas and their pathogenesis. *Brain Pathol* 2014; 24: 205–220.

15. Aleksic T, Chitnis MM, Perestenko OV, et al. Type 1 insulin-like growth factor receptor translocates to the nucleus of human tumor cells. *Cancer Res* 2010; 70: 6412–6419.

16. Asmane I, Watkin E, Alberti L, et al. Insulin-like growth factor type 1 receptor (IGF-1R) exclusive nuclear staining: a predictive biomarker for IGF-1R monoclonal antibody (Ab) therapy in sarcomas. *Eur J Cancer* 2012; 48: 3027–3035.

17. van Gaal JC, Roeffen MH, Flucke UE, et al. Simultaneous targeting of insulin-like growth factor-1 receptor and anaplastic lymphoma kinase in embryonal and alveolar rhabdomyosarcoma: a rational choice. *Eur J Cancer* 2013; 49: 3462–3470.

18. Schnarr B, Strunz K, Ohsam J, et al. Down-regulation of insulin-like growth factor-I receptor and insulin receptor substrate-1 expression in advanced human breast cancer. *Int J Cancer* 2000; 89: 506–513.

19. Eppler E, Zapf J, Bailer N, et al. IGF-I in human breast cancer: low differentiation stage is associated with decreased IGF-I content. *Eur J Endocrinol* 2002; 146: 813–821.

20. Vella V, Sciacca L, Pandini G, et al. The IGF system in thyroid cancer: new concepts. *Mol Pathol* 2001; 54: 121–124.

21. Schips L, Zigeuner R, Ratschek M, et al. Analysis of insulin-like growth factors and linsulin-like growth factor I receptor expression in renal cell carcinoma. *Am J Clin Pathol* 2004; 122: 931–937.

22. Colao A, Ferone D, Marzullo P, et al. Systemic complications of acromegaly: epidemiology, pathogenesis, and management. *Endocr Rev* 2004; 25: 102–152.