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CASE REPORT

# Expression of HIF-1, galectin-3, cox-2 and Wilms tumor-1 protein in multiple schwannomas of the conus medullaris

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**Abstract** Schwannomatosis is a clinical syndrome that requires thorough clinical and radiological assessments before the diagnosis is made. Although schwannomatosis has been reported before, all were in multiple organ sites. The authors report a case of multiple intra-dural schwannomas of the conus medullaris expressing HIF-1, galectin-3, cox-2 and Wilms tumor-1 protein; which to the best of our knowledge has not been previously reported in schwanomatosis.

**Keywords** Spinal cord · Schwannomatosis · HIF-1 · Galectin-3 · WT-1 · Cox-2

## Introduction

Schwannomatosis is a clinical syndrome that requires thorough clinical and radiological assessments before the diagnosis is made. In schwannomatosis, patients lack features characteristic of neurofibromatosis (NF) 2: bilateral vestibular schwannomata, or family history of NF2 plus unilateral acoustic neuroma or any two of meningioma, glioma, neurofibroma, schwannoma, posterior subcapsular lenticular opacities. In addition, patients must also lack features pathognomonic of NF1: café au lait patches, two or more Lisch nodules of the iris, two or more neurofibromata or one plexiform neurofibroma or a first degree relative with NF1 [1, 2]. The diagnostic criteria for schwannomatosis are listed in Table 1 [1]. Extensive work has been carried out to either link or separate the clinically distinct Schwannomatosis from Neurofibromatosis [3–5].

Multiple spinal Schwannommatosis account for 5% of all spinal tumours, occur most frequently in the cervical region, followed by the lumbo-sacral and lastly the thoraco-lumbar regions [5–7]. The tumours usually arise from the Schwann cells of the neurilemma of spinal nerve roots hence, the term neurilemmoma or schwannoma. Classically, schwannomas are extra-dural which grow and expand the intervertebral foramen. There have been reports of intra-dural and even intra-medullary schwannomas [8].

Hypoxia-inducible factor-1 (HIF-1) is a transcription factor that respond to hypoxia. The alpha subunit of HIF-1 is a target for prolyl hydroxylation by HIF prolyl-hydroxylase, which makes HIF-1 α a target for degradation by the E3 ubiquitin ligase complex, leading to quick degradation by the proteasome. This occurs only in normoxic conditions. In hypoxic conditions, HIF prolyl-hydroxylase is inhibited, since it utilizes oxygen as a cosubstrate. HIF-1, when stabilized by hypoxic conditions upregulates several genes to promote survival in low-oxygen conditions [9]. Galectin-3, a glycoprotein with a 31-kDa molecular weight, is a member of the beta-galactoside binding family of lectins [10]. It has been suggested to play a role in a variety of biological processes such as cell growth, cellular adhesion, cell cycle regulation, neoplastic transformation and metastasis [10]. Cyclooxygenase-2 (cox-2), an inducible enzyme pivotal in the inflammatory response, converts arachidonic acid to the prostaglandins required to initiate and maintain reactions during the inflammatory process. HIF-1 α up-regulation occurred in cox-2-overexpression [11].

Wilm's tumor-1 (WT-1), a well characterized tumor suppressor gene, was shown to be a transcriptional

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**Table 1** Diagnostic criteria for schwannomatosis [1]

| Definite | Possible |
|---|---|
| Age >30 years + two or more non-intradermal schwannomas, at least one with histologic confirmation + No evidence of vestibular tumor on high quality MRI scan and No known constitutional NF2 mutation, | Age <30 years + two or more non-intradermal schwannomas, at least one with histologic confirmation + No evidence of vestibular tumor on high quality MRI scan and No known constitutional NF2 mutation, |
| OR | OR |
| One pathologically confirmed non-vestibular schwannoma + a first degree relative who meets above criteria. | Age >45 years + two or more non-intradermal schwannomas, at least one with histologic confirmation + No symptoms of 8th nerve dysfunction and No known constitutional NF2 mutation, |
| | OR |
| | Radiolographic evidence of a non-vestibular schwannoma + a first degree relative meeting criteria for definite schwannomatosis. |

regulator with putative target genes including those for growth factors and regulators of cell division [12].

The authors present a rare case of four intra-dural schwannomas localised to the T12-L2 region of the spinal cord expressing HIF-1, galectin-3, cox-2 and Wilms tumor-1 protein; which to the best of our knowledge has not been previously reported in schwanomatosis.

## Case report

A 46 year-old male presented with 9 months of non-specific lower back pain. As part of the investigation process for the low back pain he underwent a magnetic resonance image (MRI) scan. This revealed intradural space occupying lesions in the thoraco-lumbar region. He was referred immediately to the neurosurgical department. System inquiry failed to reveal any other abnormalities in his present and past medical history. There was no family history of cutaneous lesions, eye pathology or brain tumours. On examination there was only mild hypoesthesia in the left L2 & L3 distribution and mild wasting of the left quadriceps muscle. No café au lait patches or visual abnormalities were detected on clinical examination. Of note the patient had a scaphocephaly with average intelligence.

MRI scan of the lumbar and thoracic region showed three intradural extramedullary lesion that were hyperintense on T2 and hypo-intense on T1 and enhanced vividly and homogenously (Fig. 1). The lesions varied in size and extended from the level of the T12 exit foramen to the level of L2 body. The conus medullaris and cauda equine seemed to be pushed to the right side. A non-enhanced brain and cervical + thoracic MRI was performed to look for any other lesions. None were found.

The patient underwent initially a lumbar puncture for cytology but that was negative for abnormal cells. Three days later the patient underwent T12-L2 laminoplasty and total resection of four intradural extra-medullary lesions under intra-operative electromyographic monitoring. All

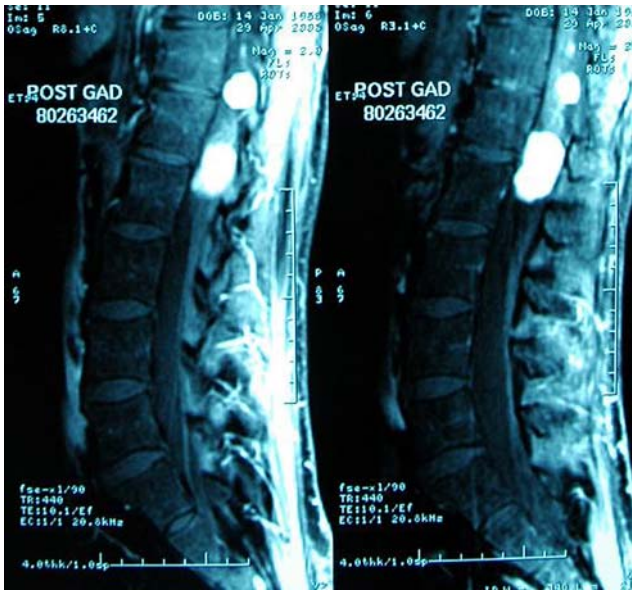


**Fig. 1** Post Gadolinium T1 weighted sagittal image. Note the homogenous and vivid enhancement

four lesions were "encapsulated", firm and attached to spinal nerve roots; T12 bilaterally, left L1 and Right L2. The T12 tumours were both fusiform and the other two globular in shape. Incision of the "capsule" and tumour debulking followed by excision of the capsule and cauterising its attachment to the spinal nerve root was utilised to resect the upper three lesions. The fourth was resected en-bloc after dissecting it free from its attachment to the right L2 nerve root. The patient suffered no neurological deficits post-operatively. The tumour capsules were sequentially dissected free from nerve roots then excised en bloc and the attachment to the nerve root cauterised with bipolar diathermy. No nerve roots were sacrificed.

Histologically, the tumour was composed of spindle to ovoid cells with ovoid or angulated nuclei closely arrayed in intermingled bundles with hyper and hypocellular areas (Fig. 2a). The hyper cellular areas composed of spindle

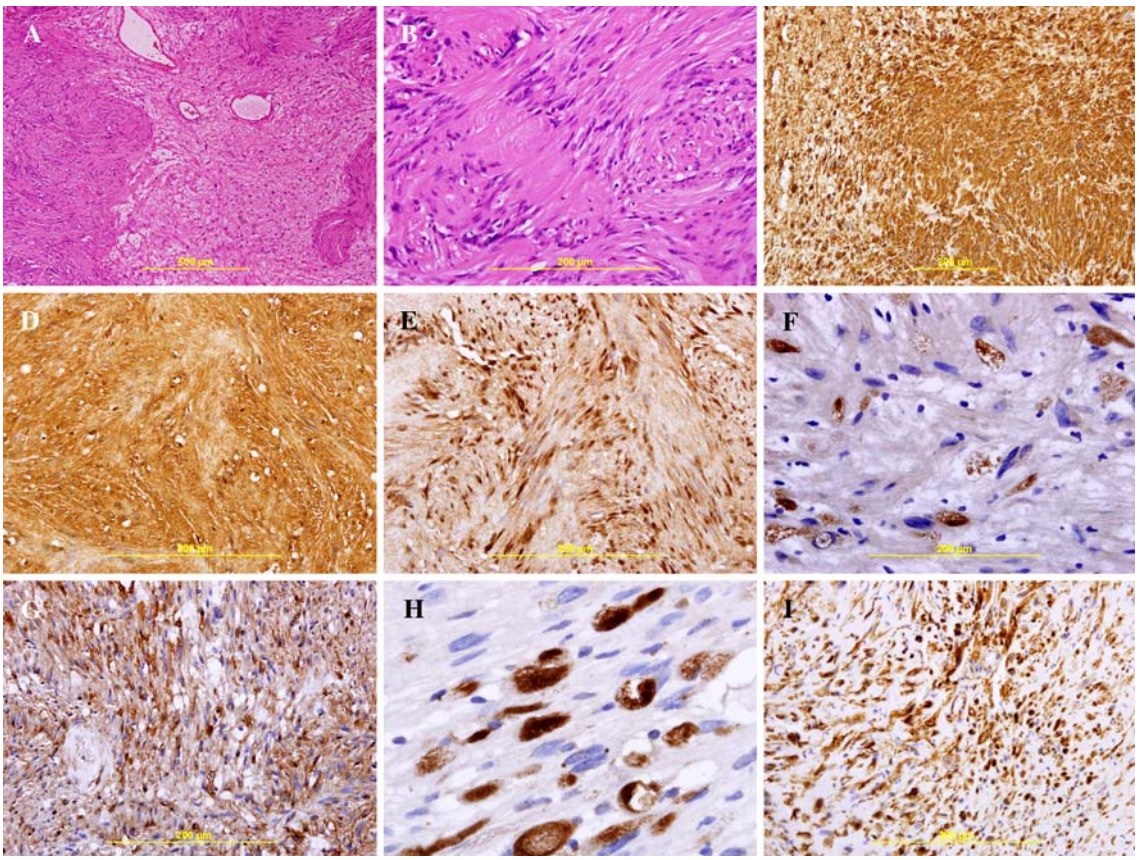


**Fig. 2** **a** Showing hypercellular and hypocellular areas, H&E. **b** Showing the characteristic palisading of the tumor cells in schwannoma, H&E. **c** Showing nuclear and cytoplasmic immunoreactivity of tumor cells to S100, streptavidin–biotin immunoperoxidase. **d** Showing cytoplasmic immunoreactivity of tumor cells to HHF-35, streptavidin–biotin immunoperoxidase. **e** Showing cytoplasmic immunoreactivity of tumor cells to neuron specific enolase, streptavidin–biotin immunoperoxidase. **f** Showing cytoplasmic immunoreactivity of tumor cells to cox-2, streptavidin–biotin immunoperoxidase. **g** Showing cytoplasmic immunoreactivity of tumor cells to galectin-3, streptavidin–biotin immunoperoxidase. **h** Showing cytoplasmic immunoreactivity of tumor cells to HIF-1, streptavidin–biotin immunoperoxidase. **i** Showing cytoplasmic and nuclear immunoreactivity of tumor cells to WT-1 protein, streptavidin–biotin immunoperoxidase

cells often arranged in a palisading fashion (Antoni A pattern) (Fig. 2b) or in an organoid arrangement while in the hypocellular areas the tumor cells are separated by abundant oedematous fluid forming cystic spaces (Antoni B pattern). Many hyalinised blood vessels were discernible.

The cells of the examined tissue were phenotyped immunohistochemically by the streptavidin–biotin method using antibodies (DAKO) all diluted to 1:50. The tumor cells were uniformly S100 (Fig. 2c), HHF35 (Fig. 2d), neuron-specific enolase (Fig. 2e), cox-2 (Fig. 2f), galectin-3 (Fig. 2g), HIF-1 (Fig. 2h), WT-1 protein (Fig. 2i) positive. The tumor cells showed no immunoreactivity to epithelial membrane antigen, cytokeratin, desmin, smooth muscle actin, glial fibrillary acidic protein, HMB-45 and synaptophysin.

Post-operatively the patient had no added neurological deficits and was mobilised on day four post-operatively. A formal ophthalmologic review failed to show any abnormalities in the iris or retina. A follow up MRI of the thoraco-lumbar area pre-and post Gadolinium showed two tiny spots of enhancement at the level of the T12 nerve roots corresponding to the area of attachment of the tumours. No other abnormal intra-dural enhancement was seen. The patient was discharged on day 12 postoperatively, fully independent and self-caring. A firm thoraco-lumbar brace was applied for 6 weeks.

## Discussion

Guidelines for the diagnosis of Schwannomatosis were proposed by MacCollin et al. in 2005 [1] based on the largest combined patient cohorts presenting with features of non-NF neurilemmoma. The typical age of presentation is over 30 years. Symptoms range from non-specific regional pain to neurologic deficit due to neural compromise. The radiological investigation of choice is MRI scanning which should typically cover the whole neural

axis. Complete surgical resection is the treatment of choice and is associated with long-term tumour free survival. The rationale of complete surgical excision (which usually entails resection of the harbouring nerve root) as opposed to enucleation within the capsule has been resolved recently. Kuo et al. [13] described the light microscopic features of ten acoustic schwannomata and concluded that there is no true capsule. Hasegawa and co-workers [14] further confirmed these findings studying spinal schwannomas capsules under the electron microscope. They concluded that there is an intermix of nerve fibres and tumour tissue within a so called capsule therefore, enucleation retains the risk of recurrence, but may provide a better functional outcome, especially with the use microscopic surgery. In our case the patient had total resection of all four lesions with a nerve sparing technique.

The clinical differential diagnoses include ependymoma and metastatic carcinoma. Ependymoma is usually intramedullary single lesion which can be easily differentiated from schwannoma by histologic examination. Moreover, metastatic carcinoma can be differentiated by histologic examination too. Immunohistochemical examination is helpful in solving the differential diagnosis since schwannoma is usually immunoreactive to S100 which is usually negative in ependymoma and metastatic carcinoma while epithelial markers are positive in metastatic carcinoma. In addition, ependymoma shows immunoreactivity to glial fibrillary acidic protein which is negative in schwannoma.

The expression of HIF-1, galectin-3, cox-2 and Wilms tumor-1 protein in neoplastic cells of present neoplasms is highly interesting and suggest a possible role in their development. In the present case, the neoplastic cells show cytoplasmic immunoreactivity for HIF-1. HIF-1 is a transcription factor composed of HIF-1$\alpha$ which dimerize with a constitutively expressed $\beta$ subunit and subsequently bind to hypoxia response elements in the promoters of target genes [9]. HIF-1$\alpha$ protein expression in cells is regulated by a variety of stimuli, including changes in cellular oxygen concentration, growth factors, oncogenic activation, or loss of tumor suppressor function. Under normal oxygen tension, the $\alpha$ subunits are hydroxylated at conserved prolyl and asparaginyl residues and are targeted for degradation by the Von Hippel-Lindau (VHL) ubiquitin E3 ligase complex. In hypoxia, inhibition of hydroxylation results in stabilization of HIF-1$\alpha$ and leads to transcriptional activation of target genes [15]. Loss or inactivation of the tumor suppressor VHL leads to accumulation of HIF in schwann cells and drives the production of various hypoxia inducible mRNAs including the mRNAs encoding vascular endothelial growth factor (VEGF), platelet-derived growth factor B (PDGF B), erythropoietin (EPO), galectin-3, WT-1 protein, and transforming growth factor alpha (TGFa). VEGF and PDGF B are paracrine growth factors that stimulate endothelial cells and pericytes, respectively and leading to vascular proliferation and proliferation of schwann cells hence, growth of the neoplasm [15]. It is noteworthy to mention here that there are multiple foci of vascular proliferation in the current neoplasms. In addition, the neoplastic cells show strong and diffuse nuclear and cytoplasmic staining for galectin-3 suggesting over expression of galectin-3 gene. Up-regulation of galectin-3 gene is mediated predominantly by HIF-1$\alpha$ [9]. Zeng et al., demonstrates that HIF-1 regulates galectin-3 expression by interacting with hypoxia regulatory elements in the promoter region [16]. Galectin-3 over expression inhibits apoptosis and may promote neoplastic transformation of schwann cells [10].

Moreover, the neoplastic cells show cytoplasmic immunostaining for cox-2. HIF-1$\alpha$ up-regulation occurred in cox-2 over expression [11]. Both cox-2 and HIF-1 up-regulation promote vascular proliferation and tumor growth.

Furthermore, the neoplastic cells show diffuse and strong nuclear and cytoplasmic staining for WT-1 protein suggesting over expression of WT-1 gene. Over expression of WT-1 protein in schwann cells is possibly due to the autocrine effect of HIF-1 which activates the transcription of WT-1 gene [15]. WT-1 protein is a transcriptional regulator with putative target genes including those for growth factors and regulators of cell division [12]. We believe that the expression of HIF-1, galectin-3, cox-2 and Wilms tumor-1 protein in neoplastic cells may be essential for the development of schwanomatosis in our case.

Recurrence or developing new tumours on follow up may occur [5]. However, there is no recurrence in our cases after 1 year of follow-up. Some patients show a familial tendency though the genetic coding does not show NF2 typical mutations [17]. Some reports advocate the use of adjunct post-operative radiotherapy or radiosurgery, but not in the case of completely excised lesions [18]. Post-operative radiotherapy is planned 3 months postoperatively after healing of the laminoplasty for this patient.

## Conclusion

Schwannomatosis is as yet, a clinical syndrome, which requires thorough clinical and radiological assessments before making the diagnosis. The long term prognosis appears to be good and recurrence after total surgical excision is uncommon. The expression of HIF-1, galectin-3, cox-2 and Wilms tumor-1 protein in neoplastic cells may be essential for the development of schwanomatosis.

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