



# Utility of Nestin immunohistochemistry in the diagnosis of granular cell tumor

## Granüler hücreli tümör tanısında Nestin immunohistokimyasının kullanımı

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### Abstract

**Aim:** Granular cell tumors (GCTs) show neuroectodermal differentiation. Morphologically, a wide variety of mesenchymal tumors may have granular cell appearance. Nestin, which is an intermediate filament protein expressed in undifferentiated cells during central nerves system development and its tumors. We aim to determine the diagnostic utility of Nestin in diagnosis of GCTs.

**Methods:** Nestin immunohistochemistry applied to GCT cases and other mesenchymal tumors which may have granular cytoplasm and the major differential diagnostic consideration of GCT. A total of 21 GCT from different tissues (including 7 in the esophagus, 8 originating from skin tissues, 4 in the tongue, 2 in the vocal cord), 17 gastrointestinal stromal tumor in the gastrointestinal tract, 8 leiomyoma (5 in the esophagus and 3 originating from skin tissues), 4 schwannoma (1 in the esophagus and 3 originating from skin tissues), subcutaneous mesenchymal tumors ( including 7 neurofibroma, 5 fibroma, 15 dermatofibroma), 20 melanocytic nevi, 15 gastric xanthomas and 15 xanthelasma of the skin were included the study.

**Results:** Nestin positivity was detected in all GCTs. Additionally, strong Nestin positivity was seen in all gastrointestinal stromal tumors, schwannoma and neurofibroma cases. However, Nestin was negative in all leiomyoma, fibroma, dermatofibroma, melanocytic nevi, gastric xanthoma and xanthelasma cases.

**Conclusion:** The study showed that Nestin immunohistochemistry has limitation in distinction of GCT from tumors arising from neural cell lineage such as gastrointestinal stromal tumor, schwannoma and neurofibroma; however, Nestin as a neural marker provides an evidence to neural origin of GCT and could be useful in distinction GCT from other mesenchymal tumors with granular cytoplasm such as leiomyoma, dermatofibroma and melanocytic nevi.

**Key words:** Nestin, granular cell tumor, neural origin

### Öz

**Amaç:** Granüler hücreli tümörler (GCTs) nöroektodermal diferansiyasyon gösterirler. Morfolojik olarak pek çok mezenkimal tümör, granüler hücreli görünüme sahip olabilir. Nestin, santral sinir sistemi gelişimi boyunca undiferansiyasyon hücrelerinde ve santral sinir sistemi tümörlerinde ekspres edilen bir intermediate filament proteindir. Çalışmamızda Nestin proteininin granüler hücreli tümör ayırıcı tanısında kullanılabilirliğinin belirlenmesi amaçlanmıştır.

**Yöntemler:** Çalışmamızda GCT tümör olguları ve granüler sitoplazmaya sahip olabilecek ve granüler hücreli tümör ayırıcı tanısında başlıca ele alınan mezenkimal tümörlere immunohistokimyasal olarak Nestin antikoru uygulanmıştır. Farklı dokulardan köken alan 21 GCT (7'si özofagus, 8'i deri, 4'ü dil, 2'si vokal korddan gelişen), 17 GIST (gastrointestinal stromal tumor- gastrointestinal kanaldan gelişen), 8 leiomyoma (5'i özofagus, 3'ü deriden gelişen), 4 schwannoma (1'i özofagus, 3'ü deri yerleşimli) ve subkutan yerleşimli mesenkimal tümörler (7 nörofibroma, 5 fibroma, 15 dermatofibroma) ile 20 melanositik nevüs, 15 gastrik ksantom ve 15 ksantalezma olgusu çalışmamıza dahil edilmiştir.

**Bulgular:** GCT'lerin tamamında Nestin pozitifliği görüldü. Ayrıca tüm GIST'ler, schwannoma ve nörofibromada Nestin pozitif olarak izlendi. Bununla birlikte tüm leiomyoma, fibroma, dermatofibroma, melanositik nevüs, gastrik ksantom ve ksantalezma olgularında Nestin ekspresyonu görülmedi.

**Sonuç:** Çalışmamız Nestin immunohistokimyasının GCT'ün ayırıcı tanısında özellikle GIST, Schwannoma ve nörofibroma gibi nöral orijinli tümörlerde sınırlı olduğunu göstermektedir. Bununla birlikte nöral bir marker olan Nestin GCT'ün nöral kökenine dair bir kanıt sunmaktadır. Nestin, GCT 'ün granüler sitoplazmaya sahip leiomyoma, dermatofibroma gibi diğer mezenkimal tümörler ve melanositik nevüsten ayırımında kullanılabilir.

**Anahtar kelimeler:** Nestin, granular hücreli tümör, nöral orijin

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Ethics Committee Approval: Ethics committee approval was not obtained because the study was retrospective.

Etik Kurul Onayı: Çalışma retrospektif olduğu için etik kurul onayı alınmamıştır.

Conflict of Interest: No conflict of interest was declared by the author.

Çıkar Çatışması: Yazar çıkar çatışması bildirmemiştir.

Financial Disclosure: The authors declared that this study has received no financial support.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Geliş Tarihi / Received: 25.06.2018

Kabul Tarihi / Accepted: 01.09.2018

Yayın Tarihi / Published: 30.11.2018

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## Introduction

Granular cell tumor (GCT) is a rare and usually benign tumor having neuroectodermal differentiation. About 2 % of the cases can present a malignant course [1]. While GCTs have been reported in various parts of the body such as the skin and the viscera, they most frequently occur in the head and the neck regions. Around 40 % of the cases are found in the tongue while the skin and subcutaneous tissue share one third of the cases. Other sites such as the esophagus, the stomach, the larynx, the bronchus, the uvea, soft tissue and the pituitary stalk may also be involved. GCTs are usually poorly circumscribed and non-encapsulated. However, encapsulated GCTs can occur, especially the skin and the subcutaneous tissue.

Histologic features of GCT are quite distinctive with abundant granular-appearing of tumor cells. However, granular cell changes due to lysosome accumulation can be observed in a variety of neoplasms and they might closely resemble one another [2]. Therefore, a wide range of tumor and reactive pathologies such as xanthoma and trauma associated changes should be considered in differential diagnosis of GCTs.

Currently, the hypothesis of the neural origin of GCT is widely accepted. Immunohistochemical studies also showed Schwann cell origin of GCT through the positive identification of S-100 protein [3-5]. Recently, Nestin expression has been shown in GCT of gastrointestinal (GI) tract [6, 7]. Nestin protein was firstly discovered in the developing nervous system of mice. Nestin is considered to be a marker of neural stem and skeletal muscle progenitor cells and tumors related to such cell lineages, although its expression in other cell types is under investigation [8]. It has been detected in many kinds of tumors, especially in tumors derived from the central nervous system such as gliomas, medulloepitheliomas, primitive neuroectodermal tumors, meningiomas [9].

The purpose of this study was to investigate the diagnostic benefit of Nestin immunohistochemistry as a neural tissue marker in the diagnosis of GCTs.

## Material and methods

### Patients and clinical data

This retrospective study consisted of 21 cases of GCT diagnosed at Baskent University, Faculty of Medicine, Department of Pathology between 2003 and 2017. Written consent and ethical approval could not be taken due to the retrospective design of the study.

The diagnosis of GCT cases have been detected from the archives of pathology report collected via electronic files on computer. Demographic data including age, gender and sites was noted by using the patient's medical records from the archives of the pathology, retrospectively.

The location of 21 GCT cases were from diverse anatomical sites. We categorized the patients into three groups according to their locations (the GI tract, the skin and the head and neck lesions). Seven cases of GCT were from gastrointestinal tract (the esophagus), eight cases of GCT were from the skin (three on the extremities, one on the vulva, one on the breast, one on the abdominal surface, two on the face) and six cases of GCT were from the head and neck region (four on the tongue, two on the vocal cord).

To investigate the specificity of Nestin, a total number of 76 cases of other mesenchymal tumors which are considered mostly in the differential diagnosis of GCTs and 15 cases of

gastric xanthomas and 15 cases of xanthelasma from the skin were also applied Nestin immunohistochemistry.

Histologic types of tumor which are considered mostly in the differential diagnosis of GCTs was determined according to literature [2]. This mesenchymal tumor group were also consisted of tumors which are located similar site of GCTs. Pathology reports were reviewed and mesenchymal tumor group were determined from archives of the pathology department between 2003 and 2017. In the mesenchymal tumor group, tumor affected the GI tract includes five leiomyoma of the esophagus, one schwannoma of the stomach and 17 gastrointestinal stromal tumor (GIST) cases. Tumors affected the skin and the soft tissue includes three leiomyoma, three schwannoma, seven neurofibroma, five fibroma, 15 dermatofibroma and 20 melanocytic nevi. Gastric xanthomas (n=15) and xanthelasma (n=15) were also studied. Among them, two leiomyoma of the esophagus, one leiomyoma of the skin, one melanocytic nevi, all gastric xanthomas and xanthelasma of the skin have granular cell appearance.

Demographic and clinicopathologic features of patients are given in table I.

### Pathological analysis

All H&E-stained slides were reviewed by a single pathologist and the diagnosis was confirmed. Immunohistochemical staining for S-100 protein, CD68 had been used for the confirmation of the diagnosis of GCT before. Representative samples with GCT were selected for immunohistochemical analysis with Nestin. To investigate the specificity of Nestin, other tumors which are considered in the differential diagnosis of GCTs were also applied Nestin immunohistochemistry.

### Immunohistochemical staining

Formalin-fixed paraffin-embedded tissue was used for the immunohistochemical reactions. Three- $\mu$ m thick sections were obtained from the formalin-fixed, paraffin-embedded tissue blocks mounted on positively charged glass slides, and dried overnight at room temperature. Sections were de-paraffinized in xylene and in graded ethanol and placed in 0.5% hydrogen peroxide in methanol for 5 minutes to block endogenous peroxidase activity. Antigen retrieval was carried out by incubation in 0.01 M citrate buffer (pH 6.0) for 15 minutes in a microwave oven. Sections were removed and put in phosphate-buffered saline for 15 minutes, following rinsed thoroughly in deionized water. The sections were exposed to the primary antibody for 60 minutes at room temperature. The standard streptavidin-biotin-peroxidase complex method was used for Nestin antibody, 1:100 dilution; (Santa Cruz-sc-71665), by employing di-amino-benzidine as the chromogen.

### Statistical analysis

Continuous variables were expressed as mean  $\pm$  standard deviation, and median value if necessary. Categorical variables were expressed as frequencies with percentages.

## Results

The histological features were similar in all GCTs. At low magnification, tumors were composed of sheets or nests of plump, round or slightly ovoid cells with abundant eosinophilic granular cytoplasm with small, round, centrally located uniform pyknotic nuclei (Figure 1). Histochemically, tumor cells were characterized by globular and diffuse periodic acid-Schiff positivity of the cytoplasm, which remained after diastase digestion. The following primary antibodies were used to confirm diagnosis: S100 and CD68. All of the cases of GCTs were positive for S-100 and CD-68. Nestin positivity was also

detected in the cytoplasm of tumor cell with diffuse and strong staining intensity (Figure 2).

In mesenchymal tumor group, strong Nestin positivity was seen in all GIST, schwannoma and neurofibroma. Nestin was negative in all leiomyoma, fibroma, dermatofibroma, melanocytic nevi, gastric xanthoma and xanthalesma. Nestin was also positive at the endothelium of vessels in both study group. The results of the immunohistochemical studies on these tumors are summarized in Table 2.

Pathology*	Anatomic site	Location*	Sex F/M	Age (year)	Size (mm)
Granular cell tumor (21)	Gastrointestinal	Esophagus (7)	4/3	42.7	7.71
	Skin	Leg (3), vulva (1), breast (1), abdominal (1), face (2)	4/4	46.3	11.2
	Head&neck	Tongue (4) Vocal cord (2)	5/1	54 46	11 4.5
Leiomyoma (8)	Gastrointestinal	Esophagus (5)	3/2	54	11.0
	Skin	Leg (3)	3/0	62	13
Schwannoma (4)	Gastrointestinal	Stomach (1)	1/0	18	9
	Skin	Arm (2), face (1)	1/2	39	10
GIST (17)	Gastrointestinal	Stomach (9), duodenum (5), liver (3)	9/8	55.9	73
Neurofibroma (7)	Skin	Extremity (7)	4/3	46.3	12
Fibroma (5)	Skin	Scalp (2), extremity (2), lip (1)	3/2	54.8	13
Melanocytic nevi (20)	Skin			49.4	7
Dermatofibroma (15)	Skin	Hand (1), trunk (5), extremity (9)	11/4	47.3	9
Xanthoma (15)	Gastrointestinal	Stomach (15)	8/7	51	5
Xantholesma (15)	Skin	Eyelid (10), face (3), extremity (2)	9/6	55	6

Table 1. Demographic and clinical features of the patients.

\*(n), F: Female, M: Male, GIST: Gastrointestinal stromal tumor

### Discussion

In the current study, we found that Nestin was uniformly expressed in GCTs which were obtained from various tissues. Similar to us, Parfitt et al. [6] demonstrated the presence of Nestin expression in GCT before. Their results were related to the GCT originated only from the GI tract. In the present study, the majority of the GCT was from the GI tract, the cutaneous tissue and less commonly from the head and the neck regions. In the GI tract, GCTs are preferentially found in the esophagus, followed by the colon and the rectum and the perianal region [7]. Similar to the literature, we found that majority of the GCT cases were localized in the esophagus in the GI tract.

Histologically, all GCTs were composed of well-demarcated proliferations of pale-stained polygonal cells with the characteristic eosinophilic granular cytoplasm and an oval nucleus. The granules stain positive with PAS but are resistant to diastase. The diagnosis was verified using immunohistochemical staining with S100 and CD68. S100 is the most widely used

antibody which supports the neural nature of the GCTs. Another antibody is CD-68 which is usually positive in the GCTs. CD68 positivity is attributed to an intracytoplasmic accumulation of phagolysosomes and does not reflect the histiocytic origin of the tumor [5]. In a wide variety of both benign and malignant tumors, they may show focally or extensively granular cell change [2]. Granular cell differentiation is due to an increased number of secondary lysosomes and hallmarked by the presence of abundant eosinophilic granularity of cytoplasm. In the differential diagnosis of GCT, we consider leiomyoma [10], schwannoma [11, 12], GIST [6] and xanthoma in GI tract, leiomyoma [13, 14], neurofibroma, dermatofibroma, melanocytic nevi [15] and xanthalesma in the skin lesions and fibroma in the oral lesions.

Table 2. Immunohistochemical Nestin expression in GCT and other tumors.

Tumor*	Nestin Positive*	Negative*	Location
GCT (21)	21	--	GI tract, cutaneous , head and neck
GIST (17)	17	--	GI tract
Schwannoma (4)	4	--	GI tract, cutaneous
Leiomyoma (8)	--	8	GI tract, cutaneous
Neurofibroma (7)	7	--	Cutaneous
Fibroma (5)	--	5	Cutaneous
Melanocytic nevi (20)	--	20	Cutaneous
Dermatofibroma (15)	--	15	Cutaneous
Xanthoma (15)	--	15	GI tract, cutaneous
Xanthalesma (15)	--	15	Cutaneous

\*: (n)

Figure 1. Tumors showing sheets or nests of plump, round or slightly ovoid cells with abundant eosinophilic granular cytoplasm with small, round, centrally located uniform pyknotic nuclei.

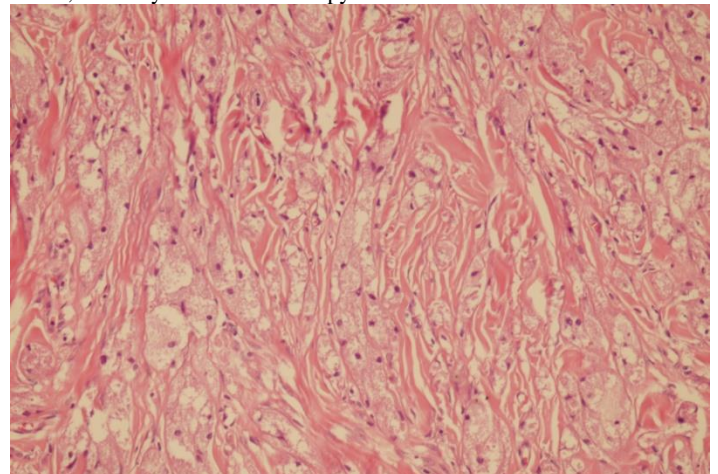
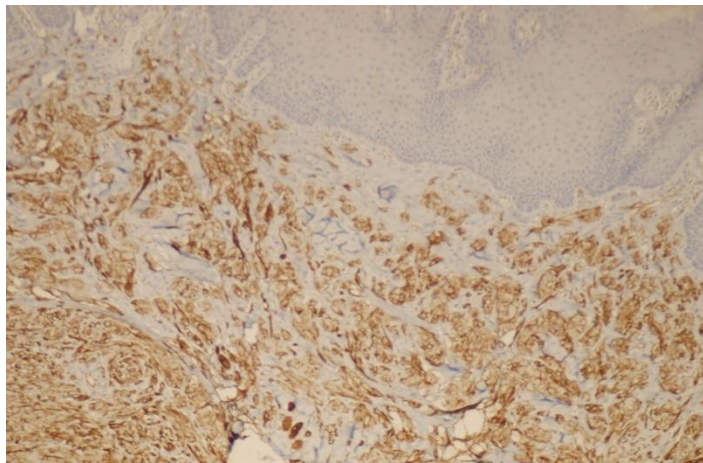


Figure 2. Nestin positivity in the cytoplasm of tumor cell with diffuse and strong staining intensity.





In our study, Nestin was positive in GISTs, schwannomas and neurofibromas. Similar to us, Sarlomo et al. [11] have shown Nestin expression in GIST and GI Schwannomas. In literature there are conflicting results on Nestin expression in schwannoma. In the present study, we demonstrated Nestin positivity in both GI and skin schwannoma (1 the esophagus and 3 the skin). On the other hand, Tsujima et al. [16] have reported no evidence of Nestin expression in schwannoma which developed in the GI tract but they showed strong immunoreactivity for Nestin in all their GIST cases. However, their study includes only one case of Schwannoma in the GI tract. A study of large series of cases presented by Hou et al. [17] have showed that Nestin expression was variably positive in thirty-three cases of GI schwannomas (78.8%, 26/33). In another study, Shimada et al. [18] reported weak Nestin expression in 10/10 schwannomas from soft tissue. On the basis of these results, we thought that further studies including more patients need to clarify the Nestin expression in schwannomas. However, our results indicated that Nestin expression could not be used to distinguish GCT from GIST, Schwannomas and neurofibroma.

In addition, our results showed that Nestin may be helpful in separating GCT from leiomyoma. We found that Nestin was uniformly negative in all leiomyomas. Nestin is known to be expressed in skeletal muscle progenitor cells, but is down-regulated on cellular differentiation [19-21]. Similar to us, studies showed that Nestin expression has not been identified in GI leiomyomas [11, 16] and leiomyomas arises soft tissue [8]. Actually, GCTs are can be distinguished from leiomyomas by immunohistochemical staining with smooth muscle actin (SMA) and desmin. While SMA and desmin reactions confirm the smooth muscle origin of the cells, negative staining with Nestin also suggests that they are unlikely to be of neural origin in leiomyomas. On the other hand, in contrast to benign muscle tumors (i.e. leiomyomas), authors have found that malignant tumors with muscle differentiation, rhabdomyosarcoma and leiomyosarcoma, showed strong Nestin expression in the majority of cases [18]. Authors have thought that Nestin expression may correlate with malignant transformation of the myogenic tumors.

Beside leiomyoma and schwannoma, we have evaluated Nestin expression in other skin lesions such as dermatofibroma, melanocytic nevi and xanthelasma. Although most of them are benign but diagnostic difficulty may arise for lesions with xanthomatous cells. In the study, none of the cases of dermatofibroma, melanocytic nevi and xanthelasma showed Nestin expression. Therefore we thought that Nestin can help to differentiate GCT from the melanocytic nevi and dermatofibroma. Similar to us authors found that the melanocytic nevi, even dysplastic nevi, showed no or weak staining with

Nestin. However increased Nestin expression has been shown to be associated with aggressive melanoma features [22, 23]. Similarly, while diffuse positive Nestin expression in dermatofibrosarcoma protuberans cases, a partial positive reaction was reported in dermatofibroma cases [24].

GCT is mostly benign, although in 1% to 3% of cases, a malignant development can occur. The histologic features such as high mitotic index and a cellular/nuclear pleomorphism are related to malignancy. Initially, because of non-neoplastic nature of the GCT, it has been considered, as a degenerative process that resulting from trauma or as a storage disorder involving histiocytes, in the etiology. In the present study Nestin expression was not detected in histiocytes rich lesion such as xanthomas, xanthelasma, dermatofibromas but GCTs. Therefore, we thought that Nestin can be useful in distinguishing the GCT from the histiocytic lesions and Nestin positivity provide an evidence for neural origin of the GCTs other than histiocytic origin.

To confirm the neural origin of GCT, authors have investigated several molecules such as p75, Calretinin, Protein gene product 9.5 (PGP 9.5), Inhibin-alpha, Galectin-3, HBME-1 and Sox10 in GCTs [25-32]. Among those antibodies, Inhibin-alpha, Galectin-3 and HMBE are not only isolated from the neural tissues but their expression has been reported in the neural tumors, such as schwannoma, neurofibroma, ganglioneuroma and malignant peripheral nerve sheath tumor, before [25-27]. On the other hand P75, Calretinin, PGP 9.5 and SOX10 have been originally isolated from neural tissues and thought to be specific for neural and nerve sheath differentiation [28-32]. However, these proteins are also expressed in many non-neural and non-nerve sheath tumors [23]. Nestin seems to be more specific for supporting the neural origin of the GCTs but its expression in other cell types is under investigation [3]. We also demonstrated that Nestin expression was seen in neuronal lineage tumors such as GISTs, schwannoma, neurofibroma.

Due to the rarity of GCT, our study has limited cases. Another limitation of our study is that our case series includes only benign tumors because of the GCTs are rarely malignant. Therefore, we also consider only benign tumors in differential diagnosis of GCT.

However the present study demonstrated that GCTs which are originated from varies tissue showed strong and diffuse positive Nestin expression.

In conclusion, based on the results of the present study, Nestin could be considered as a useful immunohistochemical marker for identifying the GCTs. Further studies may plan to determine the role of Nestin in differential diagnosis of malignant GCT.

## References

1. Di Tommaso L, Magrini E, Consales A, Poppi M, Pasquinelli G, Dorji T, et al. Malignant granular cell tumor of the lateral femoral cutaneous nerve: report of a case with cytogenetic analysis. *Hum Pathol.* 2002;33:1237-40.
2. Cardis MA, Ni J, Bhawan J. Granular cell differentiation: A review of the published work. *J Dermatol.* 2017;44:251-58.
3. Stefansson K, Wollmann RL. S-100 protein in granular cell tumors (granular cell myoblastomas). *Cancer.* 1982;49:1834-8.
4. Armin A, Connelly EM, Rowden G. An immunoperoxidase investigation of S-100 protein in granular cell myoblastomas: evidence for Schwann cell derivation. *Am J Clin Pathol.* 1983;79:37-44.
5. Filie AC, Lage JM, Azumi N. Immunoreactivity of S100 protein, alpha-1-antitrypsin, and CD68 in adult and congenital granular cell tumors. *Mod Pathol.* 1996;9:888-92.
6. Parfitt JR, McLean CA, Joseph MG, Streutker CJ, Al-Haddad S, Driman DK. Granular cell tumours of the gastrointestinal tract: expression of Nestin and clinicopathological evaluation of 11 patients. *Histopathology.* 2006;48:424-30

7. Singhi AD, Montgomery EA. Colorectal granular cell tumour: a clinicopathologic study of 26 cases. *Am J Surg Pathol.* 2010;34:1186-92.
8. Kishaba Y, Matsubara D, Niki T. Heterogeneous expression of nestin in myofibroblasts of various human tissues. *Pathol Int.* 2010;60:378-85
9. Sugawara K, Kurihara H, Negishi M, Saito N, Nakazato Y, Sasaki T, et al. Nestin as a marker for proliferative endothelium in gliomas. *Lab Invest.* 2002;82:345-51.
10. Bhattacharyya I, Summerlin DJ, Cohen DM, Ellis GL, Bavitz JB, Gillham LL. Granular cell leiomyoma of the oral cavity. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2006;102:353-9.
11. Sarlomo-Rikala M, Tsujimura T, Lendahl U, Miettinen M. Patterns of Nestin and other intermediate filament expression distinguish between gastrointestinal stromal tumors, leiomyomas and schwannomas. *APMIS.* 2002;110:499-507.
12. Shintaku M. Immunohistochemical localization of autophagosomal membrane-associated protein LC3 in granular cell tumor and schwannoma. *Virchows Arch.* 2011;459:315-9
13. Mentzel T, Wadden C, Fletcher CD. Granular cell change in smooth muscle tumours of skin and soft tissue. *Histopathology.* 1994;24:223-31.
14. Dobashi Y, Iwabuchi K, Nakahata J, Yanagimoto K, Kameya T. Combined clear and granular cell leiomyoma of soft tissue: evidence of transformation to a histiocytic phenotype. *Histopathology.* 1999;34:526-31.
15. El-Gamal HM, Robinson-Bostom L, Saddler KD, Pan T, Mihm MC. Compound melanocytic nevi with granular cell changes. *Am Acad Dermatol.* 2004;50:765-6
16. Tsujimura T, Makiishi-Shimobayashi C, Lundkvist J, Lendahl U, Nakasho K, Sugihara A, et al. Expression of the intermediate filament nestin in gastrointestinal stromal tumors and interstitial cells of Cajal. *Am J Pathol.* 2001;158:817-23.
17. Hou YY, Tan YS, Xu JF, Wang XN, Lu SH, Ji Y, et al. Schwannoma of the gastrointestinal tract: a clinicopathological, immunohistochemical and ultrastructural study of 33 cases. *Histopathology.* 2006;48:536-45.
18. Shimada S, Tsuzuki T, Kuroda M, Nagasaka T, Hara K, Takahashi E, et al. Nestin expression as a new marker in malignant peripheral nerve sheath tumors. *Pathol Int.* 2007;57:60-7.
19. Zimmerman L, Parr B, Lendahl U, Cunningham M, McKay R, Gavin B, et al. Independent regulatory elements in the nestin gene direct transgene expression to neural stem cells or muscle precursors. *Neuron.* 1994;12:11-24.
20. Kachinsky AM, Dominov JA, Miller JB. Myogenesis and the intermediate filament protein, nestin. *Dev Biol.* 1994;165:216-28.
21. Sejersen T, Lendahl U. Transient expression of the intermediate filament nestin during skeletal muscle development. *J Cell Sci.* 1993;106:1291-300.
22. Brychtova S, Fjuraskova M, Hlobilková A, Brychta T, Hirnak J. Nestin expression in cutaneous melanomas and melanocytic nevi. *J Cutan Pathol.* 2007;34:370-5.
23. Laga AC, Zhan Q, Weishaupt C, Ma J, Frank MH, Murphy GF. SOX2 and nestin expression in human melanoma: an immunohistochemical and experimental study. *Exp Dermatol.* 2011;20:339-45.
24. Mori T, Misago N, Yamamoto O, Toda S, Narisawa Y. Expression of nestin in dermatofibrosarcoma protuberans in comparison to dermatofibroma. *J Dermatol.* 2008;35:419-25.
25. Bellezza G, Colella R, Sidoni A, Del Sordo R, Ferri I, Cioccoloni C, et al. Immunohistochemical expression of Galectin-3 and HBME-1 in granular cell tumors: a new finding. *Histol Histopathol.* 2008;23:1127-30.
26. Bigotti G, Coli A, Del Vecchio M, Massi G. Evaluation of Galectin-3 expression by sarcomas, pseudosarcomatous and benign lesions of the soft tissues. Preliminary results of an immunohistochemical study. *J Exp Clin Cancer Res.* 2003;22:255-64.
27. Fine SW, Li M. Expression of calretinin and the alphasubunit of inhibin in granular cell tumors. *Am J Clin Pathol.* 2003;119:259-64.
28. Le BH, Boyer PJ, Lewis JE, Kapadia SB. Granular cell tumor: immunohistochemical assessment of inhibin-alpha, protein gene product 9.5, S100 protein, CD68, and Ki-67 proliferative index with clinical correlation. *Arch Pathol Lab Med.* 2004;128:771-75.
29. Andressen C, Blumcke I, Celio MR. Calcium-binding proteins: selective markers of nerve cells. *Cell Tissue Res.* 1993;271:181-208.
30. Hoang MP, Sinkre P, Albores-Saavedra J. Expression of protein gene product 9.5 in epithelioid and conventional malignant peripheral nerve sheath tumors. *Arch Pathol Lab Med.* 2001;125:1321-25.
31. Mahalingam M, LoPiccolo D, Byers HR. Expression of PGP 9.5 in granular cell nerve sheath tumors. *J Cutan Pathol.* 2001;28:282-86.
32. Heerema MG, Suurmeijer AJ. Sox10 immunohistochemistry allows the pathologist to differentiate between prototypical granular cell tumors and other granular cell lesions. *Histopathology.* 2012;61:997-9.
33. Campbell LK, Thomas JR, Lamps LW, Smoller BR, Folpe AL. Protein gene product 9.5 (PGP 9.5) is not a specific marker of neural and nerve sheath tumors: an immunohistochemical study of 95 mesenchymal neoplasms. *Mod Pathol.* 2003;16:963-69.