Abstract. Background: Neurofibromas are sporadic or associated with type 1 neurofibromatosis (NF1), with a higher risk of malignant progression. Materials and Methods: We investigated CD10 immunoexpression in 39 peripheral nerve sheath lesions. They were 18 typical, solitary, sporadic neurofibromas (group A) and 21 cases (group B) consisting of 11 NF1-associated cases, 3 malignant peripheral sheath tumors (MPNST) and 8 atypical neurofibromas. Results: CD10 immunopositivity was absent or very weak and focal in group A. On the contrary, CD10 was strongly expressed in group B, including all the MPNST and their metastases, with 95% sensitivity and 72% specificity in distinguishing between the two groups. Conclusion: CD10 is useful in the assessment of peripheral sheath tumors and could give evidence that atypical myxoid and/or diffuse neurofibromas, sometimes histologically difficult to distinguish from low-grade MPNST, represent not only a histological but also an immunohistochemical continuum with MPNST.

Neurofibromas are common benign peripheral tumors. They arise from the cutaneous and more rarely from the visceral, peripheral nerve sheath. In 90% of cases they are solitary, sporadic, localized, superficial tumors which are usually benign, with a very low probability of becoming malignant. The diffuse and plexiform neurofibromas have a close association with type 1 neurofibromatosis (NF1), an autosomal dominant disorder whose diagnostic criteria have been well established by the National Institutes of Health Consensus Development Conference (1). Nevertheless, NF1 diagnosis is sometimes difficult, especially in very young people in whom the initial presentation of the disease may be a solitary neurofibroma or in patients who have no affected family members (2).

Localized neurofibromas encountered in NF1 are usually larger than solitary neurofibromas occurring outside NF1, but histologically they are not different and embrace a spectrum from highly cellular to highly myxoid tumors and may display nuclear atypia. On the other hand, NF1-associated neurofibromas often show a plexiform or, more rarely, a diffuse growth pattern with a higher risk of malignant transformation into malignant peripheral nerve sheath tumors (MPNSTs). These represent about 5-10% of soft tissue sarcomas and are extremely aggressive.

Even the diagnosis of MPNST can be difficult, especially for low-grade lesions arising in the context of a neurofibroma, also in view of the fact that some neurofibromas show atypical, myxoid and cellular features overlapping some features of low-grade MPNST, of which they sometimes represent a histological continuum (2).

Histologically, neurofibromas consist of Schwann cells, positive for S100 immunostaining, together with perineural cells and fibroblasts, which are CD34 positive, but S100 negative. The S100 protein, the most widely utilized marker in the diagnosis of neurofibromas, does not distinguish the different types of neurofibromas and it has a poor sensitivity in MPNST (it is reduced or absent in some low-grade MPNSTs and in more than 2/3 of high-grade MPNSTs). Protein gene product (PGP) 9.5 and nestin are more sensitive than S100, although they are not specific since they are expressed in other mesenchymal neoplasias and they do not allow the early identification of progressing lesions (3, 4).

As it is important to reach a correct diagnosis and an early identification of progressing lesions, several markers involved in cell cycle regulation, such as p53, p16 and p27, have been investigated (5, 6). Nevertheless, they are not reliable markers for early detection of tumor progression (5), in fact, for example, overexpression and mutations of p53 have been reported in high-grade MPNST, but their late appearance precludes their use as predictive markers of malignancy (5, 7) and it is commonly accepted that “there is not yet sufficient information to recommend their routine use in diagnosis, which remains principally a light microscopic diagnosis” (2).
CD10 antigen, also known as neutral endo-peptidase (NEP), nephrilisine or CALLA antigen, is normally expressed in breast myoepithelial cells, in kidney, in the apical membranes of intestinal gland epithelium, in endometrial and in bone marrow stromal cells, in biliary canalicules of the liver and in mesenchymal dermal cells (fibroblasts and dendrocytes). It has been also reported in the normal myelin sheath of peripheral nerves (8). It was first identified on the precursors of B and T lymphocytes and in several hematopoietic neoplasias (B and T acute lymphoblastic leukemias, follicular lymphoma, Burkitt’s lymphoma). Recently, it was also evidenced in some non-hematopoietic neoplasias, such as renal cell carcinoma, prostatic cancer, melanoma, mesenchymal neoplasia and endometrial stromal sarcoma (8-13). To our knowledge, CD10 expression in lesions arising from the peripheral nerve sheath has still not been sufficiently investigated.

The aim of this study was to investigate CD10 immunohistochemical expression in lesions arising from the peripheral nerve sheath and to assess if any differences are present between benign, malignant and lesions at risk, both in sporadic and NF1-associated cases.

Materials and Methods

This study was retrospectively performed on 39 lesions consecutively selected between 2004 and 2007 from the Institute of Pathology of the University of Palermo (Italy), 18 of which (Group A) consisting of localized, sporadic, solitary neurofibromas, with typical features and in which the surgical excision had been curative, and 21 (Group B) consisting of MPNSTs and of neurofibromas with histological features related to a higher risk of malignant transformation, such as plexiform pattern, or partially overlapping some MPNST aspects, such as atypias or hypercellularity. In Group B, 11/21 patients had a well-known history of NF1 (8 with multiple neurofibromas with typical histological features, 3 of whom had a relapse history; 1 with atypical, myxoid neurofibroma; 1 with atypical diffuse neurofibroma; 1 was affected by MPNSTs); 10/21 patients had no known history of NF1 when they underwent surgery. Nevertheless, 4 showed atypical myxoid neurofibromas and 3 of them had a relapse history, 3/10 had plexiform neurofibromas, 1/10 had undergone surgical excision of 2 neurofibromas (1 of which had peripheral plexiform areas) and 2/10 were affected by MPNST, 1 of whom developed pulmonary metastases a year later (see Table I). Informed consent was obtained for all the patients included in the study.

We retrospectively performed an immunohistochemical assay by using the labelled streptavidin-biotin peroxidase method on formalin-fixed, paraffin-embedded serial 5 μm sections. Mouse monoclonal antibody 56C6 (diluted 1:200) was used to detect CD10 glycoprotein, and mouse monoclonal antibody CD34 (QBEnd/10 diluted 1:50) and the calcium-binding protein S100 (diluted 1:200). All the antibodies were from Novocastra (Newcastle, UK). The specimens were considered as negative when the staining was absent or present in less than 10% of the cell population.

Adventitial peri-annexial cells and vascular endothelial cells were used as positive internal control respectively for CD10 and CD34 immunostaining; small nerves and epidermal junctional melanocytes were used as positive internal control for S100 immunostaining.

Differences in the distribution of the study variables between the two groups were assessed by means of the non-parametric t-test (Mann-Whitney U-test) for the mean diameter and by means of the paired t-test for the evaluation of CD10, S100 and CD34 immunohistochemical expression.

Differences were considered statistically significant at p<0.05.

Results

The group A lesions were smaller than the group B lesions: group A mean diameter=0.84 cm (range 0.3-5 cm), 15/18 (83%) had a diameter <1 cm; group B: mean diameter=3.8 cm (range 0.3-15 cm), 14/21 (70%) had diameter >1 cm (Mann-Whitney U-test, p<0.05) (Figure 1).

Table I. Patients included in the study.

<table>
<thead>
<tr>
<th>Cases (n=39)</th>
<th>Clinical-histological diagnosis</th>
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<tr>
<td>Group A</td>
<td>18 Patients with solitary, typical neurofibroma</td>
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| Group B     | 21 11/21 Patients affected with clinically asserted NF1  
|             | 1 with low-grade MPNST  
|             | 3 with typical histological features but with history of relapse  
|             | 5 with multiple neurofibromas, with typical histological features  
|             | 1 with myxoid neurofibroma, with atypia  
|             | 1 with diffuse neurofibroma with atypia  
|             | 10/21 patients without known history of NF1  
|             | 4 with myxoid/atypical neurofibromas, 2 of whom with history of relapse  
|             | 2 with high-grade MPNSTs (one of them showing lung metastasis 1 year after)  
|             | 3 with plexiform neurofibroma  
|             | 1 with contemporary surgical resection of 2 neurofibromas (one of which with plexiform areas)  

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The immunohistochemical assay performed with S100 and CD34 showed no statistically significant difference between the two groups: S100 stained positively in all of the group A cases and in 19/21 cases (90%) of the group B cases (the 2 negative ones consisting of 2 MPNSTs). CD34 stained positively in 17/18 (94%) of group A cases and in 16/21 cases (75%) of group B cases.

In group A, CD10 stained negatively in 13/18 (73%) cases (Figure 2 a, b) and the remaining 5/18 cases (27%) showed only a weak, focal CD10 positivity (less than 15% of the cell population). On the contrary, in group B, CD10 stained positively in 19/21 (95%) cases, with a pattern of positivity ranging from strong but focal positivity highlighting the more atypical cells, such as in the atypical myxoid neurofibromas (Figure 2 c, d,) to strong and diffuse positive immunostaining in plexiform neurofibromas, in multiple and recurrent neurofibromas (Figure 3 a-d) and in MPNSTs (Figure 3 e, f). Furthermore, all the patients with recurrent neurofibromas showed CD10-positive immunostaining as early as at the first removal of the lesions.

The statistical analysis showed that CD10 expression was significantly different in the two groups (paired t-test, p<0.0001) with 95% sensitivity and 72% specificity in distinguishing group A from group B lesions. Differences were considered statistically significant at p<0.05.

The clinical presentation of NF1 is not always clear since the onset of the clinical signs is age dependent and there is a strong variability both among patients and in the same patient during different stages of the disease, with no relation between genotype and phenotype. Due to the strong inter- and intra-familial phenotypical and clinical variability, NF1 may sometimes require a long follow-up before being diagnosed with certainty. Nevertheless, the early correct diagnosis of NF1 is important because the most serious risk of NF1 is the malignant transformation of neurofibromas (mainly plexiform neurofibromas) into MPNSTs.

Table II. Mean diameter and positivity for S100, CD34, CD10 immunostaining in group A compared with group B.

<table>
<thead>
<tr>
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<th>Group A (18 lesions)</th>
<th>Group B (21 lesions)</th>
<th>P-value</th>
</tr>
</thead>
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<tr>
<td>Mean Ø diameter</td>
<td>0.84 cm</td>
<td>3.8 cm</td>
<td>0.0079a</td>
</tr>
<tr>
<td>S100+</td>
<td>18 (100%)</td>
<td>18 (90%)</td>
<td>&gt;0.05b</td>
</tr>
<tr>
<td>CD-34+</td>
<td>17 (94%)</td>
<td>15 (75%)</td>
<td>&gt;0.05b</td>
</tr>
<tr>
<td>CD-10+</td>
<td>5 (27%)</td>
<td>19 (95%)</td>
<td>&lt;0.0001b</td>
</tr>
</tbody>
</table>

*aMann-Withney U-Test (non-parametric t-test); bpaired t-test.

Figure 1. Distribution of the diameters of the lesions in the two groups.

Discussion

NF1, also known as Von Recklinghausen’s disease or peripheral neurofibromatosis, is one of the most frequent, progressive genetic diseases, with serious medical and social consequences. It is transmitted in an autosomal dominant manner in about 50% of cases and is sporadic in the remaining cases due to new mutations (small deletions or puntiform mutations) on the germinal cells of paternal origin involving the NF1 gene, mapping on the long arm of the chromosome 17 (17q11.2). The NF1 gene is an onco-suppressor gene with a probability of mutation 10-100 times greater than the mean of other genes. It encodes neurofibromin, a protein that inhibits cell proliferation by modulating the mitogenic pathway signaling through inactivation of p21-RAS. Inactivating mutations of the NF1 gene, with the production of truncated, inactive protein, determine hyperactivation of p21-RAS and proliferation of neoplastic cells.

Finally, CD10 persisted as positive in the pulmonary metastases one year later of a primary cutaneous “high-grade” MPNST positive for S100, CD34 and CD10 immunostaining, whereas S100 and CD34 expression was lost (Figure 4).
Neurofibromas showing atypical, myxoid or cellular features are sometimes difficult to distinguish from low-grade MPNSTs and many studies have been carried out with aim of finding new molecular markers (i.e. p53, CDKN2A, p16, Bcl2, growth factors, MIB-1, N-CAM) for the screening of NF1 disease and to allow the early identification of progressing lesions (5-7, 14).

Recently CD10 appeared to be involved in the transformation and/or the progression of many types of tumor, such as breast carcinoma, gastric carcinoma, melanoma and colon cancer (11, 15-17).

In our study, CD10 expression was assessed in order to evaluate if it could be useful in diagnostic and/or prognostic assessment of neurofibromas and their malignant variants. In our hands, CD10 proved to be able to distinguish group A lesions from group B lesions, with 95% sensitivity and 72% specificity. In fact, it proved to be lacking from most of the benign, localized, sporadic neurofibromas (in which the only surgical removal had been curative), and was found in all the NF1-related lesions, in the at-risk lesions (in which it was able to highlight the plexiform or atypical aspects) and in the recurrent neurofibromas (as early as at the first removal). Moreover, CD10 stained positively both in sporadic and in NF1-related MPNST and their metastases, including the cases that were negative for S100 and CD34. In this regard, even if not specific, CD10 may be useful to confirm the link between the first tumor and the second lesion, mainly when this is negative for S100 and CD34 immunostaining. To our knowledge, no studies are present in the literature on CD10 expression in neurofibromas and MPNST, so the mechanisms and the related effects need to be investigated.

Previous studies on progressing melanoma showed an increase of NEP gene transcription (the gene encoding CD10 antigen) together with coexpression of other genes involved in cell proliferation and cancerogenetic mechanisms, such as the mitogen activated protein kinase (MAPK) pathway.
apoptosis and WNT signaling inhibition and hyperexpression of the proliferation marker Ki-67. In melanoma progression, a synergistic effect of the transcription products of these genes with the transcription product of NEP gene has been hypothesized (11, 17). Of course, further studies are required to assess if the above reported observations concerning melanomas are valid for peripheral nerve sheath tumors, also in view of the hypothesis of a common neuroectodermal origin for both tumors, probably from common precursor cells of the neural crest. Furthermore, in many tumors, such as melanoma, gastric cancer and breast cancer, a relationship between CD10 expression and a potential for neoplastic

Figure 3. Group B case: multiple neurofibromas in a patient with no clinical information regarding NF1 available at the time of the histological diagnosis. CD10 immunostaining was strong and diffuse in the first lesion (a, b). In the other lesion (c, d), CD10 highlighted the peripheral areas showing a tendency for the plexiform pattern, barely evident on haematoxylin-eosin staining (a: hematoxylin-eosin x200; b: CD10 x200; c: hematoxylin-eosin x100; d: CD10 x100). e, f: Group B case: malignant peripheral nerve sheath tumor in a patient with NF1. Strong and diffuse CD10 immunostaining (e: hematoxylin-eosin x200; f: CD10 x200).
invasiveness has been observed (11, 15-18). This could be due to the similarity between CD10 and the matrix metalloproteinases (MMPs) in creating a microenvironment facilitating neoplastic invasion, as previously hypothesized by Bilalovic et al. for melanoma (11). Analogously, the stronger immunostaining at the peripheral edge of the group B lesions, often observed in our study, could support this hypothesis and suggest a potential for local invasiveness leading to increased size (as confirmed by our results), to pseudo-infiltrative deep margins and to a higher risk of local relapses, present in diffuse, myxoid and plexiform neurofibromas and in NF1-associated neurofibromas, all belonging to group B.

Whatever the mechanism and the effects related to CD10 hyperexpression, in our opinion, its diagnostic and predictive value in neurofibromas and their malignant variants cannot be ignored.

In fact, although not specific because it is expressed in other neoplasias of different origin, prompt assessment of CD10 from the first removal of the lesion may be useful in the histological assessment of MPNST. Moreover, as NF1 diagnosis can be difficult due to the incomplete clinical picture or, sometimes, to the absence of clinical data accompanying the specimen submitted for the histological examination, CD10 immunopositivity could help in distinguishing solitary, localized neurofibromas, (not requiring a follow-up because surgical excision is usually curative) from NF1 cases from atypical, plexiform and relapsing cases, where it stained positively from the first removal. Finally, in our opinion, CD10 positivity in atypical myxoid and/or diffuse neurofibroma, which is sometimes histologically difficult to distinguish from low-grade MPNST, could provide evidence for the hypothesis that these lesions could represent not only a histological but also an immunohistochemical continuum with MPNST. In fact, CD10 overexpression may be involved in the promotion of MPNST pathogenesis. Of course, progressive acquisition of several gene mutations is necessary in malignant progression. Nevertheless, in our opinion, CD10 could be useful to identify the cases for which a follow-up would be advisable. To our knowledge, this is the first study in which CD10 immunoenexpression has been investigated in the peripheral nerve sheath lesions and a therapeutic interest, for example by using NEP inhibitors [as suggested for melanomas by Velasquez et al. (17)] in targeted therapy or in adaptive T-cell therapy could be hypothesized.
References


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