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CD57 (leu-7) Expression is helpful in diagnosis of the follicular variant of papillary thyroid carcinoma

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Abstract CD57 (HNK-1) is a oligosaccharide antigen that is expressed by cells of several lineages. It is present on multipotential neuroepithelial cells during embryogenesis, and tumours of epithelial, neuroectodermal and nerve sheath origin also express CD57. Its role in the diagnosis of thyroid tumours is controversial. We have studied CD57 expression by immunohistochemistry to determine its utility in the classification of thyroid follicular lesions. Study material included 114 normal thyroid sections, 77 benign thyroid lesions (29 colloid nodules, 22 follicular adenomas, 20 cases of Hashimoto's thyroiditis and 6 of Grave's disease) and 83 thyroid carcinomas, including 31 follicular variants of papillary carcinoma. We observed CD57 positivity in 95% of thyroid carcinomas, 27% of follicular adenomas and 10% of colloid nodules. It was not expressed in the normal thyroid. CD57 expression in thyroid carcinomas was significantly different from that in normal and benign thyroid lesions ($P < 0.0001$). The follicular variant of papillary thyroid carcinoma also showed significantly higher CD57 expression than colloid nodules ($P < 0.0009$) or follicular adenomas ($P < 0.0009$). No significant difference was seen between colloid nodules and follicular adenomas. We conclude that CD57 immunohistochemistry is valuable in the classification of thyroid follicular lesions into benign and malignant groups and is also helpful in the diagnosis of the follicular variant of papillary thyroid carcinoma.

Key words CD57 (Leu-7) · Thyroid carcinoma · Follicular variant of papillary carcinoma

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Introduction

The separation of thyroid follicular lesions into benign and malignant categories is sometimes difficult. This problem is greatest in distinguishing follicular adenomas and hyperplastic colloid nodules from minimally invasive follicular carcinoma and the follicular variant of papillary carcinoma. A reliable immunohistochemical method that could aid in the diagnosis of these thyroid lesions would be useful. One candidate marker for such a method is the oligosaccharide antigen CD57 (Leu-7).

CD57 was first identified on a subset of normal lymphocytes with a natural killer activity (NK cells) [1]. Subsequently, anti-CD57 antibodies have been shown to bind specifically to myelin-associated glycoprotein [19] and to tumours arising from tissues containing myelin associated glycoprotein [2, 5, 15, 28–30]. Additional studies have demonstrated CD57 expression in thyroid tumours [6, 10, 16, 23] and other carcinomas [4, 8, 12, 18, 21, 27, 32], but only rarely in normal epithelial tissues and benign lesions, such as hyperplastic prostatic epithelium [27, 32]. Two studies [6, 10] have suggested that the expression of CD57 may be useful in the separation of thyroid follicular lesions into benign and malignant groups, and particularly in the differentiation of papillary carcinoma from benign lesions with pseudopapillae [6]. However, a third study of 66 thyroid lesions (33 benign 33 malignant) led to the conclusion that CD57 is of limited utility in the diagnosis of thyroid carcinoma [16], and a fourth study [23] suggested that CD57 immunostaining cannot be used as a specific marker of malignancy in the assessment of follicular neoplasms of the thyroid, in this case by fine needle aspiration cytology.

In view of these contradictory data regarding the role of CD57 in the diagnosis of thyroid carcinoma, we studied CD57 immunostaining in 160 thyroid lesions (77 benign, 83 malignant) and in 114 examples of normal thyroid tissue by avidin-biotin immunohistochemistry. Our goals were to resolve the contradictory results by more careful assessment of the staining than had been done previously, using semi-quantitative methods. We also wanted to examine CD57 expression specifically in the follicular variant of papillary carcinoma, which has not been previously reported.

Materials and methods

All primary thyroid carcinomas excluding medullary carcinoma resected at the University of Massachusetts Medical Center during a 5-year period (1991–1996) were obtained from the surgical pathology files. There were 83 carcinomas (71 papillary, 6 Hurthle cell, 3 follicular, 2 insular and 1 anaplastic carcinoma). Of the 71 papillary carcinomas, 31 were the follicular variant of papillary carcinoma. Similar number (77) of nonmalignant thyroid lesions received during the same period were selected from the pathology files for the study. These were 29 colloid nodules, 22 follicular adenomas, 20 cases of Hashimoto's thyroiditis and 6 cases of Graves' disease. Normal thyroid tissue samples from 81 carcinoma cases and 33 benign lesions were also evaluated. Paraffin blocks selected for immunostaining from these cases included both lesional and normal thyroid tissue, whenever possible. The number of blocks selected from each case for immunohistochemistry ranged from 1 to 3. All the slides were reviewed and the histological diagnosis was confirmed.

All thyroid resection specimens included in this study were fixed in 10% buffered formalin and routinely processed through a VIP Tissue Tek processor. Sections were cut at a thickness of 4 μm heated at 60°C for 30 min, then deparaffinized and hydrated through a series of xylenes and alcohol prior to staining. The slides were microwaved with a proprietary antigen retrieval solution (citrate buffer; BioTek Solutions, Santa Barbara, Calif.) for 5 min in an 800-W microwave oven. Following replenishment of this solution the slides were microwaved again for an additional 5 min and then allowed to cool for 20 min. Immunohistochemical staining was performed with a monoclonal antibody to CD57 (Leu7, Becton Dickinson Immunocytochemistry Systems, Mountain View, Calif.) at a dilution of 1:20 using a standard avidin/biotin complex (ABC) method as implemented on a Techmate 1000 (BioTek) automated immunostainer. The staining procedure consisted of a 45-min incubation in the primary antibody followed by brief buffer washes, and then incubation in a cocktail of biotinylated anti-mouse IgG/IgM (BioTek) for 30 min. The slides were then washed, incubated in avidin/biotin complex (BioTek) for 30 min, washed, and reacted with diaminobenzidine and hydrogen peroxide to visualize the end-product. The sections were counterstained with haematoxylin. A small cell carcinoma of the lung was used as a positive control, and for a negative control, non-immune serum was substituted for primary antibody.

Only discrete granular cytoplasmic and/or membrane staining was regarded as positive. Staining of follicular colloid in the absence of staining of the follicular epithelium was considered nonspecific and negative. Positive staining was further classified into weak, if the intensity of staining was light brown and granular and strong if the staining was medium to dark brown in colour.

Differences in CD57 immunoreactivity between groups (normal, colloid nodule, follicular adenoma, Hashimoto's thyroiditis, Graves' disease and carcinoma) overall was evaluated using the Freeman Halton extension of Fisher's exact test. Pairwise comparisons between individual groups was performed using Fisher's exact test with Bonferroni adjustment, the latter to compensate for the additive type I error that arises from multiple comparisons. The sensitivity and specificity of positive CD57 immunostaining in the diagnosis of thyroid carcinoma was calculated by standard statistical methods.

Table 1 CD57 Expression in benign and malignant thyroid lesions. *P*-value <0.0001 (CD57 expression in benign versus malignant thyroid lesions)

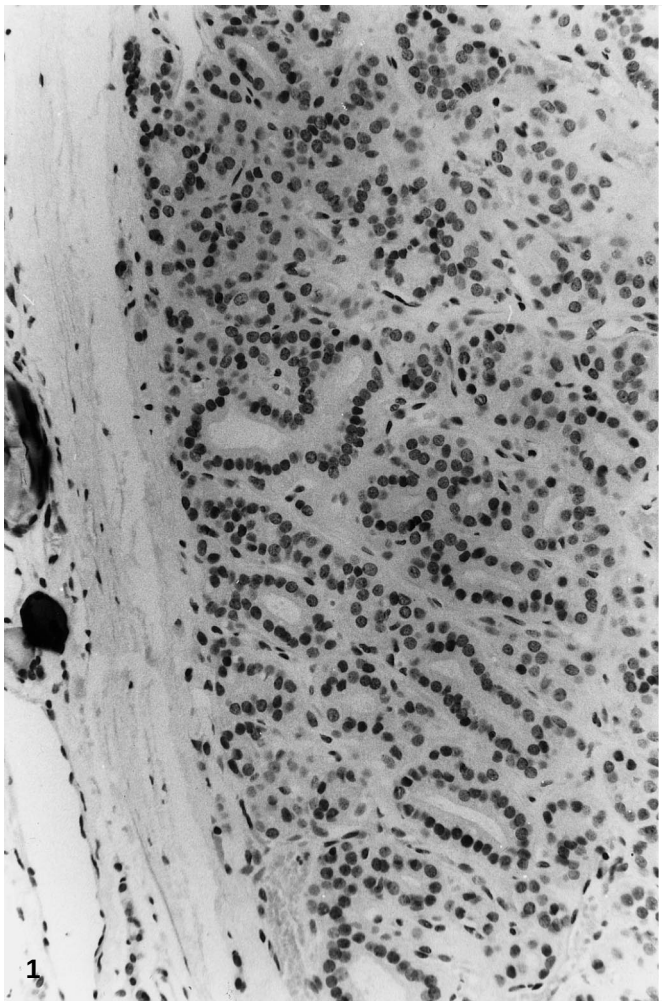
	Benign	Malignant
No. of cases (*n*)	77	83
No. CD57 positive	16	79
% CD57 positive	21	95

Results

Carcinomas showed significantly more CD57 immunostaining than normal and benign thyroid lesions ($P < 0.0001$). As shown in Table 1, 95% of carcinomas were CD57 positive, as opposed to 21% of benign lesions. When present, the CD57 staining in benign lesions was always weak. Comparison of CD57 expression within subgroups of benign and malignant lesions in Tables 2 and 3 reveals significant differences between individual categories. Of particular significance is the difference between the immunostaining of the follicular variant of papillary carcinoma and of two morphologically similar lesions with which it may be sometimes confused, follicular adenoma and colloid nodule ($P < 0.0009$). Follicular variant of papillary carcinoma consistently showed strong staining (26/31 cases), while the majority of cases of follicular adenoma (Fig. 1) and colloid nodules were CD57 negative. Focal nonspecific nuclear staining was seen in some follicular adenomas.

Table 2 Pairwise correlation of CD57 immunoreactivity in different subgroups of thyroid lesions (*NM* normal thyroid, *CN* Colloid nodule, *AD* follicular adenoma, *HT* Hashimoto's thyroiditis, *GD* Graves' disease, *CA* thyroid carcinoma, *FVPC* follicular variant of papillary carcinoma)

Diagnosis	*n*	No. CD57 +	% CD57 +	*P*-value	Bonferroni adjusted *P*-value
NM vs CA	114 vs 83	0 vs 79	0 vs 95	<0.0001	<0.0009
CN vs CA	29 vs 83	3 vs 79	10 vs 95	<0.0001	<0.0009
AD vs CA	22 vs 83	6 vs 79	27 vs 95	<0.0001	<0.0009
HT vs CA	20 vs 83	5 vs 79	25 vs 95	<0.0001	<0.0009
GD vs CA	6 vs 83	2 vs 79	33 vs 95	0.0029	0.0222
CN vs FVPC	29 vs 31	3 vs 30	10 vs 98	<0.0001	<0.0009
AD vs FVPC	22 vs 31	6 vs 30	27 vs 98	<0.0001	<0.0009
NM vs CN	114 vs 29	0 vs 3	0 vs 10	0.0064	0.0576
CN vs AD	29 vs 22	3 vs 6	10 vs 27	0.1499	Not significant



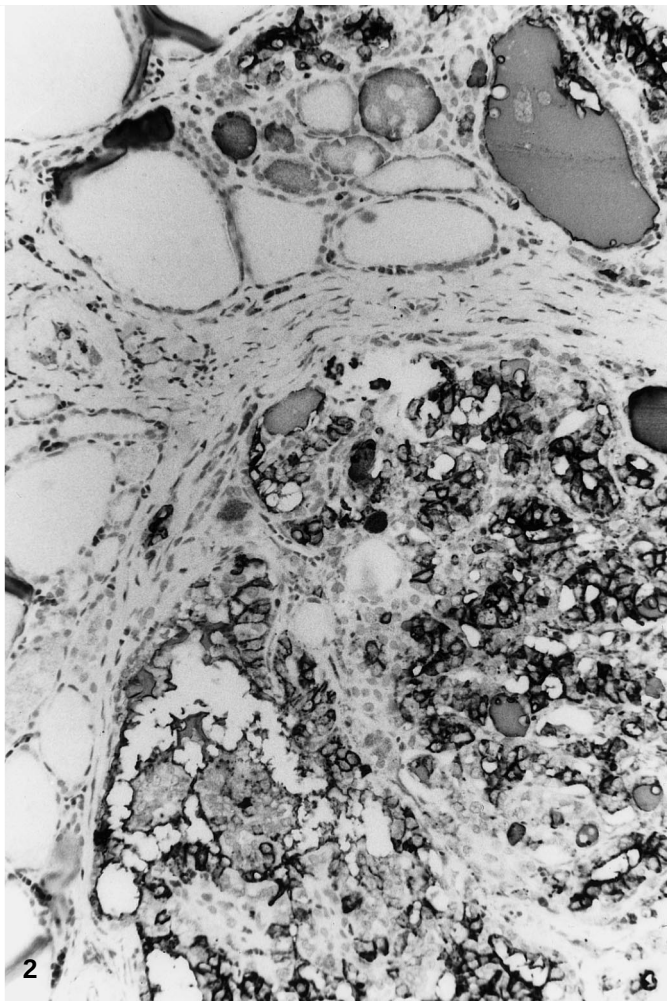


Fig. 1 Follicular adenoma showing negative CD57 immunostaining. Immunoperoxidase, ×200

Fig. 2 Papillary carcinoma showing strong positive CD57 immunostaining (bottom half). Also note positive tumour cells (top centre) interspersed between the negative staining residual normal thyroid follicles. Immunoperoxidase, ×200

Table 3 CD57 staining patterns in benign and malignant thyroid lesions

Diagnosis	*n*	CD57 immunostaining		
		Positive		Negative
		Weak	Strong	
Papillary carcinoma	40	0	38	2
Follicular variant of Papillary carcinoma	31	4	26	1
Follicular carcinoma	3	2	1	0
Hurthle cell carcinoma	6	4	2	0
Insular carcinoma	2	1	1	0
Anaplastic carcinoma	1	0	0	1
Normal	114	0	0	114
Colloid nodule	29	3	0	26
Adenoma	22	6	0	16
Hashimoto's thyroiditis	20	5	0	15
Graves' disease	6	2	0	4

To test the value of CD57 immunostaining more quantitatively in the diagnosis of malignancy in a subset of thyroid lesions with a follicular growth pattern, we performed sensitivity and specificity analysis. In 91 thyroid lesions with a follicular growth pattern (29 colloid nodules, 22 follicular adenomas, 31 follicular variants of papillary carcinoma, 3 follicular carcinomas and 6 Hurthle cell carcinomas), the sensitivity of CD57 immunostaining for the diagnosis of malignancy was 97.5% and the specificity was 82%. We also established that the predictive value of a positive test was 81% and the predictive value of a negative test was 98%.

Analysis of CD57 staining within malignant subcategories (Table 3) revealed other differences. Among the malignant lesions, all conventional type papillary carcinomas showed strong CD57 staining (Fig. 2) in a pattern similar to that seen in the follicular variant of papillary carcinoma (Fig. 3). Four out of 12 cases of other types of carcinomas, 1 follicular carcinoma, 1 insular carcinoma, and 2 Hurthle cell carcinomas, showed CD57 staining as strong as that seen in papillary carcinoma. Two cases of minimally invasive follicular carcinoma, 4 of 6 Hurthle cell carcinomas, and 1 insular carcinoma were weakly positive. The solitary case of anaplastic carcinoma did not express any CD57.

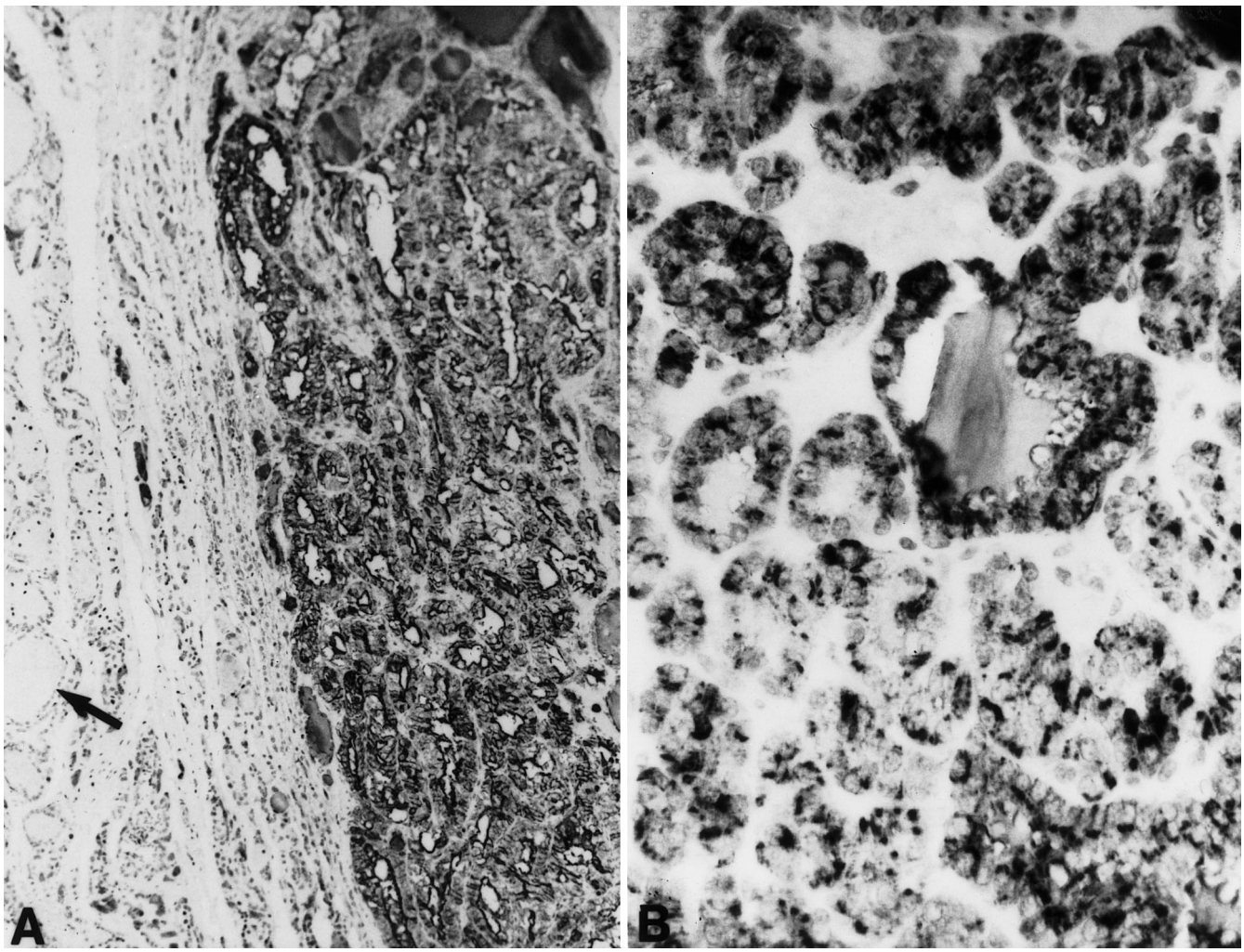


Fig. 3A, B Follicular variant of papillary carcinoma with strong positive CD57 immunostaining. **A** Peripheral portion of the tumour; note the residual normal thyroid tissue (*arrow*) is negative. Immunoperoxidase, ×100. **B** Central portion of the tumour with strong granular cytoplasmic staining of the neoplastic follicles. Immunoperoxidase, ×400

In contrast to the carcinomas, there were no statistically significant differences for CD57 staining between subcategories of benign lesions (Table 2). However, it is interesting to note that a significant fraction (21%) of the benign lesions (16/77 cases) showed some weak CD57 staining, while none of the normal thyroid samples showed any staining at all (Table 3). This is unlikely to be a false negative result, since all of the normal tissue was taken from the cases used in this study and was processed and stored similarly to the lesional tissue.

Discussion

CD57 immunostaining appears to be useful in the classification of thyroid follicular lesions into benign and malignant groups as an adjunct to H&E staining. In particular, it helps to distinguish the follicular variant of papillary carcinoma from benign follicular lesions such as colloid nodules and follicular adenoma, with which it can sometimes be confused on histology. This important comparison was not made explicitly in previous studies [6, 10], which did not consider the follicular variant of papillary carcinoma [7] as a separate group. These studies, while in general agreement with ours, are of less practical value because of this omission.

The utility of CD57 immunostaining in our hands is highly dependent on the evaluation of staining intensity. We found that although the majority of the benign lesions were CD57 negative, those that were positive stained weakly in comparison with the malignant lesions. This result is in agreement with two previous studies [6, 10], but not a third [16]. Using 66 thyroid lesions (33 benign and 33 malignant), this last study yielded the conclusion that while CD57 immunoreactivity is seen more often in thyroid carcinomas (82%) than in benign thyroid lesions (33%), it is not useful in the diagnosis of thyroid carcinoma because of the positivity in the benign lesions. However, the majority of the benign cases showed only focal, weak staining of the lesion, similar to that seen by ourselves and others [10]. The 2 of 11 cases that stained

strongly might be explained by differences in the immunohistochemical technique utilized [16]. The observation that the benign tissue that stained strongly also stained only focally suggests that the pattern and intensity of staining are additional criteria augmenting the usefulness of CD57 immunostaining distinguishing benign from malignant follicular lesions of the thyroid.

CD57 compares favourably with other markers in its utility for the classification of thyroid follicular lesions. Authors using a panel of antibodies including thyroglobulin, calcitonin, vimentin, CEA, keratin, lactoferrin and lactalbumin concluded that immunohistochemistry added little to the distinction between adenomas and carcinomas [9]. Nevertheless, the staining patterns of vimentin [22], various cytokeratins [24, 26, 31], and lactoferrin [31] have been considered helpful in differentiating benign from malignant thyroid lesions, papillary carcinomas from follicular carcinomas, papillary carcinoma from follicular neoplasms and nodular hyperplasia, or papillary and follicular carcinomas from follicular adenomas. However, none of the studies cited above demonstrates as clear-cut a distinction between benign and malignant lesions as we have shown with CD 57.

CD 57 immunostaining has also found useful application in the diagnosis of other tumours, in part because its specific monoclonal antibody recognizes a polysaccharide antigen present on a variety of polypeptides. CD57 immunostaining has been found to be useful in the identification of nerve sheath tumours [5, 11, 29], central nervous system tumours [5], normal and neoplastic neuroendocrine cells [5, 17], hyperplastic and malignant prostatic epithelium [18, 25, 27, 32] sweat gland tumours of the skin [12], Wilms' tumour, Ewing's sarcoma, and malignant melanoma [21]. Our study suggests a more definitive use for CD57 in the diagnosis of thyroid lesions than in these other tumours.

A potentially important biological implication of our study is that CD57 expression is up-regulated on malignant thyroid cells. While the function and regulation of CD57 is unknown, its importance is suggested by its presence on a spectrum of tissues, specifically, as sharing of the antigenic carbohydrate epitope between peripheral nerve sheath cell myelin-associated glycoprotein, natural killer cells [19] (where it was first identified), and glycoproteins on the surface of cells of other lineages. The nature of the polypeptide moiety of CD 57 remains to be elucidated, and may in fact differ from one cell type to another. A possible function is suggested by events of embryogenesis, during which multipotential neuroepithelial cells express an epitope recognized by the anti-CD57 monoclonal antibody. It is possible that macromolecules carrying these epitopes have a role as adhesion molecules, promoting interaction among cells and between cells and the stroma [3, 13, 14, 20]. Furthermore, the neoplastic transformation of thyroid follicular epithelium may lead to the expression of a neoantigen owing to the synthesis of a protein present on primitive endocrine cells or by way of posttranslational modification of a pre-existing protein.

Such molecular events may be the basis for our main conclusion that CD57 immunostaining is valuable in the classification of thyroid follicular lesions into benign and malignant groups. They may also help to explain our observation that the follicular variant of papillary carcinoma can be distinguished from follicular adenomas and colloid nodule with the help of CD57 immunohistochemistry.

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References

1. Abo T, Balch CM (1981) A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). J Immunol 127:1024–1029
2. Adelman LS, Wolfe HJ, DeLelis RA (1985) Immunologic cross reactivity between human neural, neuroendocrine, and T-lymphocyte antigen (abstract). J Neuropathol Exp Neurol 44: 316
3. Bronner-Fraser M (1986) Analysis of the early stages of trunk neural crest migration in avian embryos using monoclonal antibody HNK-1. Dev Biol 115:44- 55
4. Bunn PA JR, Linnoila I, Minna JD, Carney D, Gazdar AF (1985) Small cell lung cancer, endocrine cells of the fetal bronchus, and other neuroendocrine cells express the Leu-7 antigenic determinant present on natural killer cells. Blood 65:764–768
5. Caillaud J-M, Benjelloun S, Bosq J, Braham K, Lipinski M (1984) HNK-1 defined antigen detected in paraffin-embedded neuroectoderm tumours and those derived from cells of the amine precursor uptake and decarboxylation system. Cancer Res 44:4432–4439
6. Cheifetz RE, Davis NL, Robinson BW, Berean KW, LeRiche JC (1994) Differentiation of thyroid neoplasms by evaluating epithelial membrane antigen, Leu-7 antigen, epidermal growth factor receptor, and DNA content. Am J Surg 167:531–534
7. Chen KT, Rosai J (1977) Follicular variant of papillary thyroid carcinoma: a clinicopathological study of six cases. Am J Surg Pathol 1:123–130
8. Cole SPC, Mirski S, McGary RC, Cheng R, Campling BG, Roder JC (1985) Differential expression of the Leu-7 antigen on human lung tumour cells. Cancer Res 45:4285–4289
9. Davila RM, Bedrosian CWM, Silverberg AB (1988) Immunocytochemistry of thyroid in surgical and cytologic specimens. Arch Pathol Lab Med 112:51–56
10. Ghali VS, Jimenez EJS, Garcia RL (1992) Distribution of Leu-7 antigen (HNK-1) in thyroid tumours: its usefulness as a diagnostic marker for follicular and papillary carcinomas. Hum Pathol 23:21–25
11. Johnson MD, Glick AD, Davis BW (1988) Immunohistochemical evaluation of Leu-7, myelin basic protein, S100-protein, glial-fibrillary acidic-protein and LN3 immunoreactivity in nerve sheath tumours and sarcomas. Arch Pathol Lab Med 112:155–160
12. Kanitikas J, Zambruno G, Viac J, Panzini H, Thivolet J (1987) Expression of neural- tissue markers (s-100 protein and Leu-7 antigen) by sweat gland tumours of the skin. J Am Acad Dermatol 17:187–191
13. Kruse J, Mailhammer R, Wernecke H, Faissner A, Sommer I, Gorodis C, Schachner M (1984) Neural cell adhesion molecules and myelin associated glycoprotein share a common carbohydrate moiety recognized by monoclonal antibodies L2 and HNK-1. Nature 311:153–155
14. Kruse J, Keilhauer G, Faissner A, Timpl R, Schachner M (1985) The J-1 glycoprotein: A novel nervous system cell adhesion molecule of the L2/HNK-1 family. Nature 316:146–148

15. Lipinski M, Braham K, Caillaud J-M, Carlu C, Tursz S (1983) HNK-1 antibody detects an antigen expressed on neuroectodermal cells. J Exp Med 158:1775–1780
16. Loy TS, Darkow GVD, Spollen LE, Diaz-Arias AA (1994) Immunostaining for Leu-7 in the diagnosis of thyroid carcinoma. Arch Pathol Lab Med 118:172–174
17. Martin JME, Maung RT (1987) Differential immunohistochemical reactions of carcinoid tumours. Hum Pathol 18:941–945
18. May EE, Perentes E (1987) Anti-Leu7 immunoreactivity with human tumours and its value in the diagnosis of prostatic adenocarcinoma. Histopathology 11:295–304
19. McGarry RC, Helfand SL, Quaries RH, Roder JC (1983) Recognition of myelin- associated glycoprotein by monoclonal antibody HNK-1. Nature 306:376–378
20. McGarry RC, Riopelle RJ, Roder JC (1985) Accelerated regenerative neurite formation by a neuronal surface epitope reactive with the monoclonal antibody, Leu-7. Neurosci Lett 56:95–100
21. Michels S, Swanson PE, Robb JA, Wick MR (1987) Leu-7 in small cell neoplasms: an immunohistochemical study with ultrastructural correlations. Cancer 60:2958–2964
22. Miettinen M, Franssila K, Lehto V-P, Paasivuo R, Virtanen I (1984) Expression of intermediate filament proteins in thyroid gland and thyroid tumours. Lab Invest 50:262–270
23. Ostrowski ML, Brown RW, Wheeler TM, Green LK, Schaffner DL (1995) Leu-7 immunoreactivity in cytologic specimens of thyroid lesions, with emphasis on follicular neoplasms. Diagn Cytopathol 12:297–302
24. Permanetter W, Nathrath WBJ, Lohrs U (1982) Immunohistochemical analysis of thyroglobulin and keratin in benign and malignant thyroid tumours. Virchows Arch [A] 398:221–228
25. Pinkerton PH (1985) The natural-killer-cell-associated HNK-1 (Leu-7) antibody reacts with hypertrophic and malignant prostatic epithelium. Cancer 56:289–293
26. Raphael SJ, Mckeown-Eyssen, Asa SL (1994) High molecular weight cytokeratin and cytokeratin-19 in the diagnosis of thyroid tumours. Mod Pathol 7:295–300
27. Rusthoven JJ, Robinson JB, Kolin A, Pinkerton PH (1985) The natural killer cell associated HNK-1 (Leu-7) antibody reacts with hypertrophic and malignant prostatic epithelium. Cancer 56:289–293
28. Schuller-Petrovic S, Gebhart W, Lassman H, Rumpold H, Kraft D (1983) A shared antigenic determinant between natural killer cells and nervous tissue. Nature 306:179–81
29. Smolle J, Walter GF, Kerl H (1985) Myelin-associated glycoprotein in neurogenic tumours of the skin: an immunohistochemical study using Leu-7 monoclonal antibody. Arch Dermatol Res 277:141–142
30. Swanson PE, Manivel JC, Wick MR (1987) Immunoreactivity for Leu-7 in neurofibrosarcoma and other spindle cell sarcoma of soft tissue. Am J Pathol 126:546–560
31. Tucarri G, Barresi G (1985) Immunohistochemical demonstration of lactoferrin in follicular adenoma and thyroid carcinomas. Virchows Arch [A] 406:67–74
32. Wahab ZA, Wright GL (1985) Monoclonal antibody (anti-Leu7) directed against natural killer cells reacts with normal, benign and malignant prostate tissues. Int J Cancer 36:677–683